PREPARATION AND APPLICATION OF CARBON BASED NANOCOMPOSITES FOR PRECONCENTRATION OF SELECTED EMERGING ORGANIC POLLUTANTS IN ENVIRONMENTAL SAMPLES

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by

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DEDICATION

I dedicate this dissertation to my mother Miss Ramasela Elizabeth Lekota.

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ABSTRACT

Water is the most vital substance for all life on earth and a valuable resource for human civilization. Access to clean and affordable water is the most basic goal for human beings and it is a major global challenge especially in developing countries such as South Africa. It is fundamentally important to implement basic water treatment methods in developing countries where water infrastructure is very poor or even of nonexistence. The increasingly stringent water quality standards compounded by emerging contaminants have brought new scrutiny to the existing water treatment and distribution systems widely established in developing countries. Current water purification technologies and infrastructure are now in their demanding stage to provide enough water of highest quality to satisfy human and environmental needs. Common analytical techniques for determination of organic pollutants in water system include high performance liquid chromatography and gas chromatography both coupled with different detectors. However, the analytes are normally incorporated in complex matrices which makes it difficult detected them directly. Therefore, the aim of this study was to develop sample preparation methodologies for preconcentration of selected emerging pollutants in wastewater samples prior chromatographic determination.

Firstly, MgO-ZnO@CNFs and Al_2O_3-Fe_3O_4@CNFs nanocomposites were both synthesised by simple solution mixing-evaporation method. The structural and morphological properties of the material were characterized using Fourier transform infrared spectroscopy (FTIR), X-ray powder diffraction (XRD), scanning electron microscope/energy-dispersive spectroscopy (SEM/EDS), transmission electron microscope (TEM) and Brunauer–Emmett–Teller (BET).

Secondly, Fe_3O_4-Al_2O_3@CNFs and Al_2O_3-Fe_3O_4@CNFs nanocomposites were used as adsorbents in ultrasound assisted dispersive magnetic solid phase microextraction (UA-UA-DMSPME) and ultrasound assisted dispersive solid phase microextraction (UA-DSPME) methods. These methods were used for preconcentration of 17-beta estradiol (E2) and carbamazepine (CBZ) from wastewater. The analytes of interest were quantified using high performance liquid chromatography with a diode array detector (HPLC-DAD). Several parameters such as pH, mass of adsorbent, extraction time and eluent volume were first screened out by using fractional factorial design approach then further optimised was conducted using by using Box-Behnken and central composite design.

Under optimized conditions, the linear dynamic ranges were LOQ-1000 ng mL^{-1} and LOQ-800 ng mL^{-1} with correlation coefficients 0.9922-0.9951 for UA-DMSPME and UA-

DSPME methods, respectively. For UA-DMSPME/HPLC-DAD procedure (determination of E2), the preconcentration factor (PF), limit of detection (LOD) and limit of quantification (LOQ) were found to be 334, 0.25 µg L^{-1} and 0.83 µg L^{-1}, respectively. While for UA-DSPME/HPLC-DAD method (quantification of CBZ), the PF, LOD and LOQ 490, 0.08 µg L^{-1} and 0.29 µg L^{-1}, respectively. The developed methods were successfully applied for the determination and quantification of E2 and CBZ in spiked wastewater samples and the recoveries ranged from 97.8-102% respectively. These findings indicated that both methods were efficient in the extraction, preconcetration and quantification of E2 and CBZ from complex matrices

LIST OF PUBLICATIONS AND CONFERENCES

The findings of this minor dissertation resulted in two submitted manuscripts that form part of this dissertation:

1. **Lekota, M.W**. Mpupa, A., Dimpe, K.M. & Nomngongo, P. N. Ultrasound assisted dispersive magnetic solid phase microextraction based on Fe_3O_4-Al_2O_3@CNFs nanocomposite coupled with high performance liquid chromatography for extraction, preconcentration and quantification of 17-beta estradiol (E2) in wastewater. Journal of Chromatography A (under review).
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2. **Lekota, M.W.** Dimpe, K.M. & Nomngongo, P. N. MgO-ZnO/carbon nanofiber nanocomposite as an adsorbent for ultrasound assisted dispersive solid-phase microextraction of carbamazepine (CBZ) from wastewater prior to high performance liquid chromatographic detection. **International Workshop on Porous Materials and their Applications (IWPMA-2018) held in Casa Toscana Pretoria, South Africa 13 -14 September 2018**

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LIST OF ABBREVIATIONS

Abbreviation	Definition
ANOVA	Analysis Of Variance
BET	Brunauer–Emmett–Teller
CBZ	Carbamazepine
CNFs	Carbon Nanofibers
DAD	Diode Array Detector
DDLLME	Dispersive Liquid-Liquid Microextraction
DMSPME	Dispersive Magnetic Solid Phase Microextraction
DSPME	Dispersive Solid Phase Microextraction
E2	17-β Estradiol
EPs	Emerging Pollutants
ET	Extraction Time
EV	Eluent Volume
FTIR	Fourier Transform Infrared Spectroscopy
FWHM	Full Width at Half Maximum
GC	Gas Chromatography
HIV	Human Immunodeficiency Virus
HPLC	High Performance Liquid Chromatography
IUPAC	International Union of Pure and Applied Chemistry
JCPDS	Joint Committee on Powder Diffraction Standards
LC-MS	Liquid Chromatography Mass Spectroscopy
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
LPME	Liquid Phase Microextraction

MA	Mass of Adsorbent
MAE	Microwave-Assisted Extraction
MCNT	Multi-Wall Carbon nanotube
OFAT	One Factor at a Time
PDA	Photo Diode Array
PF	Preconcentration Factor
PLE	Pressurised Liquid Extraction
POPs	Persistent Organic Pollutants
RSA	Republic of South Africa
RSD	Relative Standard Deviation
RSM	Response Surface Methodology
SEM/EDS	Scanning Electron Microscope/ Energy-dispersive spectroscopy
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
SRT	Solids Retention Time
TEM	Transmission Electron Microscope
UA-DMSPME	Ultrasound Assisted Dispersive Magnetic Solid Phase Microextraction
UA-DSPME	Ultrasound Assisted Dispersive Solid Phase Microextraction
UAEME	Ultrasound Assisted Emulsification Microextraction
USA	United States of America
USD	United States Dollar
XRD	X-ray Diffraction

CHAPTER ONE:
INTRODUCTION

1.1 BACKGROUND

About 884 million people on the globe lack access to safe and clean water which is about one eighth of the world's population (Grimshaw, 2009). African and Asian women suffer the most because they walk an average distance of six kilometres just to get some water supplies (Grimshaw, 2009). Around 3.6 million people die every year due to water related diseases and ninety eight per cent of water related deaths take place in developing countries (Grimshaw, 2009). Eighty four per cent of the water related children between the ages of zero to fourteen account for deaths. Diarrhoea accounts for forty three per cent of these water related deaths (Prüss-Üstün, Bos, Gore, & Bartram, 2008). One of the human rights of every South African is the right of access to clean water and basic sanitation, but climate change and water pollution impacted by the human race is slowly depriving South Africans this right and also endangering all life on earth as a whole (Muller, 2011). Due to global warming the earth is becoming increasingly uncertain, volatile and warmer climate. A 2 ^{o}C increase in global temperatures means a 4 ^{o}C increase for South Africa, the results of this is less rain in the western half of the country and potentially more intense flood events in the east (WWF-SA, 2016). This is already happening since the Western Cape went through longest or most persistent drought duration in its history (Botai, Botai, de Wit, Ncongwane, & Adeola, 2017).

The demand for industrialisation and human infrastructure are the major reasons for nature destabilisation and environmental pollution. Intensive industrialisation results in the contamination of the land and aquatic environments via the release of toxic heavy metals, organic and inorganic materials which can accumulate in living organisms and cause several disorders and diseases (Pradeep & Anshup, 2009). These materials include pharmaceuticals, heavy metals, personal care products, disinfection by-products, insecticides, herbicides, hormones, dyes and gasoline additives, among others. When present in very low concentrations in well balanced water some of these pollutants can be harmless except for pharmaceuticals which are designed to function in extremely low doses (Pradeep & Anshup, 2009). However, some of these toxic materials, when present in high concentrations can contaminate the water which can be very harmful when consumed (Nagajyoti, Lee, & Sreekanth, 2010). Among these pollutants, pharmaceuticals are considered to be the most common and lethal (Pronczuk & Damstra, 2008).

Pharmaceutical pollutants are drugs and their metabolites, which enter different environments such as water, soil and air in which they accumulate and thus disturbing their nature, most of these pollutants are considered to be organic (Larsson, 2014). Organic pollutants are compounds that are made up of carbon and hydrogen and smaller amounts of other various other atoms such as chlorine, nitrogen sulphur to name but few. Organic pollutants can be very toxic and cause several environmental problems (Rao and Rao 1997). Types of organic pollutants include oxygen-demanding wastes, synthetic and natural organic compounds, among others. Nevertheless, the most common organic pollutants are referred to as persistent organic pollutants (POPs). These types of organic pollutants are very toxic and hazardous to the environment (Pronczuk & Damstra, 2008). The persistent nature of these compounds allows them to build up in human, animal and plant tissues and can be found in food and when consumed they can cause serious problems such as cancer and disruption of the immune system (Vacha et al., 2013).

The major problem associated with a lot of organic pollutants especially POPs is that they are highly resistant to any type of chemical, biological, and photolytic degradation; and they are bioaccumulative (Lombardi, 2015). Furthermore, they can travel very long distances from the discharge point by utilising many forms of transportation such as wind and water. These characteristics make these group of pollutants to pose potential health defects on humans and the environment at large (Degg, 2015). Organic pollutants such as hormones, antibiotics, pesticides, herbicides and other emerging pollutants have proven to be lipophilic and can also accumulate in environmental samples such as soils, water and sediments and are highly harmful pollutants to living species (Vicent, Caminal, Eljarrat, & Barceló, 2013). Soil is the world's most important environmental compartment and it is the ultimate source of food. Through their accumulation in it, they can be indirectly consumed by humans, plants and animals through the food and water (Xie, Paau, Li, Xiao, & Choi, 2010). In addition, since they are not easily degraded, they can also travel from species to species throughout the food chain accumulating in the bigger animals as they eat the smaller ones (Xie et al., 2010). Exposure to organic pharmaceutical pollutants can cause diseases or abnormalities in a number of wildlife species, including certain kinds of fish, birds, and mammals (Pronczuk Damstra, 2008). Once consumed by humans they cause reproductive, developmental, behavioural, neurologic, endocrine, and immunologic adverse health effects (Pronczuk & Damstra, 2008).

1.1.1 Pharmaceuticals

Pharmaceuticals are chemical or biomolecule substances that can be organic or inorganic and are manufactured by the pharmaceutical industries to either activate or inhibit certain function of a target in order to trigger a biological response that helps the administrator. There is a range of different types of pharmaceuticals drugs meant to treat different viruses, diseases and disorders. Hence the pharmaceutical industry is one of the biggest in the world with a revenue 1,105 billion USD (Statistica, 2016).

In the past three to four decades, the environmental pollution studies focused on major or priority pollutants such as pesticides and industrial intermediates. As environmental research growth expanded and advancement in technology other significant pollutants that existed in our environment were discovered (Bhandari, 2009). These pollutants include pharmaceuticals, personal care products, endocrine disrupters, perfluorinated pollutants, surfactants, steroids, organometallic pollutants, poly-aromatic hydrocarbons, plasticisers and nanoparticles (Gros, Petrović, & Barceló, 2006). Out of these emerging pollutants, pharmaceutical pollutants seem to be one of the most widespread and common. In the past, research on pharmaceutical environmental pollution was almost entirely focused on the release of harmful chemicals in large amounts or high concentration, that has changed in present days it has changed to also include compounds present in low concentrations because they can also be harmful even more than those available in higher concentration (Larsen, Lienert, Joss, & Siegrist, 2004). Trends from 1985 to 1999 showed the production of medicine is growing rapidly in value as and this growth is more than the world's income. Most of this growth is concentrated in well developed countries which manufacture these medicines and sell them to developing countries (World Health Organization 2004). According to (United Nations Office on Dugs and Crimes 2017), about 255 million people have some kind of drug use disorder. Non-prescription medicines are easy to obtain from any pharmaceutical shop and they made about USD 316 billion in the year 2000 (World Health Organization 2004).

These massive productions of medicine release harmful chemicals in to the environment and thus polluting it. Before even finding their way into the market, the synthetic process, by-products, cleaning, extraction and purification of these drugs make their way into the environment penetrating the water, soil, sediments and air (Snyder, 2008). Even after their use, whether used for agriculture, in animals or human beings, they are not fully consumed in the body or in plants and they can form by-products which are then later excreted via urine, faeces, sweat, vomit, roots of plants and even leaves (Larsen et al., 2004). The release of drugs from

the human body depends upon numerous factors such as how the parent drug transforms in the body, the structure of metabolites that are formed by enzyme and the amount drug as well as the metabolites that are excreted out of the body (McEneff, Schmid, & Quinn, 2015). These conclusions are made possible through a study called pharmacokinetics which studies how drugs are absorbed, distributed, metabolised and released from the body (Duffull, 2012).

Human activity such as industrialisation is the major influence that is responsible for the release of pharmaceuticals into the environment (Daughton & Ternes, 1999). Some of the release of the pharmaceuticals is involuntary; these include excreting them through the body or washing them down the drain. Some of these pharmaceuticals are produced in the body for example hormones, cytokines, enzymes, vaccines and antibodies (Almeida, Amaral, & Lobão, 2011). One of the major problems associated with pharmaceuticals is that they are not easily detected by most conventional water treatment methods; the evidence of this is the presence of high concentration of pharmaceuticals in water that is already treated (Snyder, 2008). The ineffectiveness of the removal of these pollutants poses a real health problem to aquatic organisms, plants and public health. Pharmaceuticals are used extensively throughout the globe and each and every year new ones are introduced into the market which explains their contribution to the constant increase of their environmental presence as well as their active metabolites in the environment mostly in water streams (Daughton & Ternes, 1999). Another problem associated with pharmaceutical pollutants is that some of them are persistent. Even though some of them are not, their continuous introduction into the environment due to their extensive use means they are considered to be pseudo-persistent (Ebele, Abou-Elwafa Abdallah, & Harrad, 2017). Researchers have found that pseudo-persistent pharmaceuticals have higher potential to be persistent in the environment than other organic pollutants such as pesticides, because they can overpower or resist natural environmental processes such as biodegradation, photo-degradation and particulate sorption due to their continuous introduction into the environment. Hence, pharmaceuticals which cannot deteriorate would eventually and effectively behave as persistent pollutants because of their constant presence in the environment (Houtman, van Oostveen, Brouwer, Lamoree, & Legler, 2004).

The major concern regarding toxicological entanglement of pharmaceuticals is that their sole purpose is to optimise their biological activity at extremely low dosage and to target specific metabolic, enzymatic, or cell-signalling mechanisms. These pharmaceuticals can also be active in non-target organisms posing further health defects (Fabbri & Franzellitti, 2016). Pharmaceutical pollutants can also interact with endocrine systems that can result in undesired effects or disruption of homeostasis. According to the World Health Organization endocrine

disruptors are defined as ‘exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an organism, its progeny or sub-population’ (Ebele et al., 2017). There is a variety of endocrine disrupting pharmaceuticals including; sex hormones, antibiotics, veterinary growth hormones and steroids (Huerta et al., 2016).

Pharmaceutical residues do not only persist in aquatic systems but also in soils and sediment. For example, the pharmaceutical compounds carbamazepine, lamotrigine (which are anti-epileptic drugs), sildenafil (found in Viagra) and sulfapyridine (which is an antibiotic) were found to be resistant to biodegradation in soil, and they also accumulated in soils that were irrigated with treated wastewater containing these compounds (Grossberger, Hadar, Borch, & Chefetz, 2014). The pharmaceuticals accumulate in wastewater treatment plants. This has led to investigations into the use of waste water for irrigation which has been found to contribute to pharmaceutical contamination of soil and groundwater (Rodríguez-Navas et al., 2013). This means that the use of recycled domestic wastewater for irrigation can lead to persistent contamination of the soil, sediments as well as groundwater by pharmaceuticals (Murdoch, 2015).

1.1.1.1 Anticonvulsants

Anticonvulsants are a diverse group of pharmaceutical drugs that are primarily used for treating patients with epileptic seizures. Seizures are a sudden, uncontrollable, brief and excessive electrical disturbance in a group of cells in the brain (Livingston, Pauli, & Pruce, 2001). The tendency of these electrical discharges to reoccur frequently is a disorder called epilepsy (Livingston et al., 2001). Anticonvulsants are not only used for the treatment of epilepsy but they can also treat bipolar disorder, neuropathic pain syndromes and borderline personality disorder (Keck, McElroy, & Strakowski, 1998; Oldham et al., 2005; Rogawski & Löscher, 2004). Anticonvulsants work by suppressing the excessive rapid firing of electrical discharges during seizures (Macdonald, 1988). Seizure are well known to spread within the brain, anticonvulsants are also used to stop this from happening (Harden, 1994). Treatment is normally required when a person has two or more seizures; this is because more seizures are likely to occur. Treatment is not initiated after a single seizure case because there is some evidence this may not improve in the long term prognosis (Gschwind & Seeck, 2016; Wiebe, Téllez-Zenteno, & Shapiro, 2008).

Since there are so many anticonvulsants available in the market today, it may be difficult to choose the right on, but generally this is guided by several factors such as type of epileptic seizure, age, health status (such as people with other long term diseases e.g. HIV, diabetes etc.) and the risk of adverse effects. The firstly prescribed anticonvulsant is sodium valproate for patients with general epilepsy syndromes. However, in pregnant women a low dosage of about less than 200 mg is prescribed for every day. Since sodium valproate is a teratogenic drug, carbamazepine is preferred for such cases (Forsberg & Wide, 2011). In the case of partial seizures lamotrigine or carbamazepine are the anticonvulsant drugs that are preferred for initial treatment (Marson et al., 2007)

There is about 43 704 000 people living with epilepsy and this number is only reported from 108 countries that cover about 85.4% of the population on earth. Out of this number, 75% of them reside in developing countries with poor resources and hence have little to no medical treatments, about 7.7% of total epileptic patients live in Africa (Meinardi, Scott, Reis, & Sander, 2001; Ngugi, Bottomley, Kleinschmidt, Sander, & Newton, 2010). With such numbers come large markets with large industrial productions as well as massive revenue hence the anticonvulsant market was valued at 4,200 billion US dollars in 2012 and it expected to reach 5.4 billion US dollars by 2022. In 2013 alone, about 4.3 million adults and 750,000 children between the ages of 0 to17 were diagnosed with epilepsy (Gohil & Enhoffer, 2014).

The rising ubiquity of epilepsy increases the demand for third-generation anticonvulsant drugs, such as Zebinix, Sunovion, Stedesa, Trobalt, and Potig, which is expected to drive epileptic anticonvulsant drug market up over the forecast period (Martin-Diaz et al., 2009). This is bad news for humans, animals and the environment as a whole because these drugs are hard to biodegrade and they are made to perform in low concentrations. These drugs are released in to the environment via industrial effluents, smoke chimneys and by faeces, urination and vomiting by humans who consume them (Martin-Diaz et al., 2009). They then accumulate in the soil, water and plants that are then consumed by plants, animals and humans. These drugs can also accumulate in the body leading to several diseases. Hence there is a need to detect them at these small concentrations in different media and remove them from the consumable resources (Martin-Diaz et al., 2009).

1.1.1.1.1 Carbamazepine (CBZ*)*

Carbamazepine ($C_{15}H_{12}N_2O$) is a tricyclic compound that is used for treatment of epilepsy and sold under the brand name of tegretol. This is the first anticonvulsant drug ever used for treatment of partial seizure and it showed excellent results without secondary generalization (Okuma, 2002). Primarily, CBZ is used to treat patients with epilepsy and neuropathic pain. The effectiveness of this drug is comparable with that of phenytoin and valproate with the added advantage of being able to treat partial epilepsy but its weakness comes in the treatment of absence seizures and myoclonic seizures as it is not effective as compared to its counterparts (Nolan, Marson, Pulman, & Tudur Smith, 2013; Tudur Smith, Marson, Clough, & Williamson, 2002)

Although it is not completely known how CBZ treats epilepsy or nerve pain, research indicates that it decreases nerve impulses that may lead to seizures (Tudur Smith et al., 2002). It reduces polysynaptic responses and stops the activity of the sodium channel that is stimulated by batrachotoxin in cultured neuroblastoma cells, this helps to reduce abnormal electrical activity between the nerve cells., and this is regarded as the main mechanism of all the anticonvulsant (Calandra, Mauro, Cutugno, & Martino, 2016). CBZ has an IUPAC name of 5-H dibenz[b, f]azepine-5-carboxamide, it is an iminostilbene derivative that is chemically related to the tricyclic antidepressants and has strong structural similarities with phenytoin (Fig. 1.1).

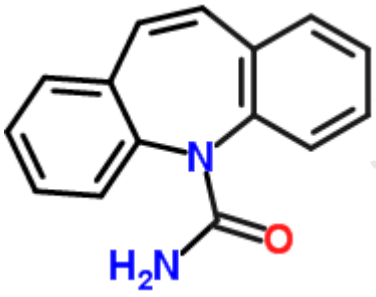

Fig. 1.1: Structure of carbamazepine obtained from (Zhang, Zhu, Qiao, Chen, & Gao, 2017)

Although CBZ is one on the most widely used anticonvulsants in the world, it has its own disadvantages, with toxicity being at the top of that list (Okuma, 2002). Its side effects include swollen tongue, loss of balance, vomiting, rapid weight gain, hallucinations, little or no urinating among others (Oldham et al., 2005). Its long term effects include modification of plasma lipids, sex hormones concentration changes, production of hyponatremia, increased appetite which may lead to weight gain, reduction of the number of white blood cells in the body and induce several allergic reaction (Keränen & Sivenius, 1983; Oldham et al., 2005).

Carbamazepine can also interact with other anti-epileptic drugs or with other drugs in the system, such as antibiotics, contraceptives and calcium channel blockers (Emilien & Maloteaux, 1998) and induces its own hepatic metabolism and this can generate a variety of other intermediate drugs (Ambrósio et al., 2002).

When CBZ is administrated in the body over long period, it can lead to some serious health problems. Both adult males and females can suffer from sexual dysfunction and reproductive endocrine disorders when taking CBZ (Najafi, Ansari, Zare, Fatehi, & Sonbolestan, 2012). This drug can also reduce the potency of sperm and production of abnormal sperms in males (Asadi-Pooya, Farazdaghi, & Ashjazadeh, 2015). In females, this drug can produce menstrual disorders, polycystic ovaries and eventually infertility (Isojärvi, Laatikainen, Pakarinen, Juntunen, & Myllylä, 1995). In anti-epileptic drug induced androgen deficiency, it is believed that CBZ is the major cause, this is done by inducing the synthesis of sex hormone binding globulin, the glycoprotein which binds androgens and estrogens, and this reduces the number of hormones that are bound loosely (Najafi et al., 2012). Enough CBZ in the blood can lead to an increase in serum 17-beta estradiol levels, which result in the reduction of testosterone production which is done through the negative feedback loop (Löfgren, Tapanainen, Koivunen, Pakarinen, & Isojärvi, 2006).

Carbamazepine is not only toxic to patients medically treated by it; it can also pose as threat to other organisms and the environment at large (Andreozzi, Marotta, Pinto, & Pollio, 2002). This drug is release into the environment from pharmaceutical industries, hospitals and from households into the air, water and soil which can make its way up in to plants which are consumed by other animals (Ebele et al., 2017). All pharmaceutical drugs are made to work at very small doses but the major problem with CBZ is that it is very resistant to biological degradation (persistent in nature) so it builds up in the water and the soil even in the body of another organism which can lead to multiple health defects (Miao, Yang, & Metcalfe, 2005). Another major problem is that most anticonvulsants cannot be effectively removed by most conventional water treatment methods so it also builds up in water treatment plants on example is the high concentration of to 709 ng/l found in one of the waste water treatment plants in New Zealand (Murdoch, 2015). Hence, there is a need to find new analytical methods that can be able to detect such pollutants and remove them from our drinking water.

1.1.2 Hormones

Hormones are chemicals synthesised and secreted by specific cells called endocrine glands, these hormones act as messengers that transmit signals from one cell to another (Henry, 1855). They are transported by circulatory systems to specifically target organs at a distance for regulation of physiology and behaviour in multicellular organisms (Tata, 2005). When the hormone reaches the target cell it binds to it and transmit the message, this can change several aspect of the cell including cell growth, metabolism, or other functions (Nelson, 2010). Due to their diverse chemical structures, hormones are classified according to their different properties such as solubility, the transmitted signal, chemical make-up and location of their receptors (Henry et al., 1855). Using the chemical structures, hormones can be grouped into three classes, steroid, amines and peptides. In this work, much focus is on steroid hormones more specifically oestrogens. Steroid hormones are vital chemicals responsible for proper function of the body by mediating various crucial functions including anti-inflammatory agents to regulate events during pregnancy (Brown & Fishman, 2000). Estrogens are hormones that are found in the adrenal cortex and gonads that are responsible for growth and function of female secondary sex organs. Estrone, estradiol, estriol, and estetrol are four major naturally occurring female sex hormones (Brown & Fishman, 2000) and this work focuses on 17-β estradiol

1.1.1.1 17-Beta estradiol (E2)

17-Beta estradiol ($C_{18}H_{24}O_2$) is a type of steroid sex hormone that is essential for sexual characteristics in women. It is crucial for the regulation and maintenance of fecundity, estrous and the female menstrual cycle. The E2 is responsible for characteristics such as development and growth of breasts, broadening of the hips and the distribution of fat that is responsible for feminine pattern which also plays a critical role in the way women are shaped (DeRuiter 2002). This hormone is involved in the production and maintenance of tissues that are responsible for female reproductive system, tissues such as the mammary glands, uterus endometrium, cervix, and vulva in the period of pubescence, maturity, and gestation (Ryan, 1982). The structure of E2 is a 17-beta isomer of estradiol with carbon 18 that is aromatised with OH group at 3-beta and 17-beta position (Fig. 1.2).

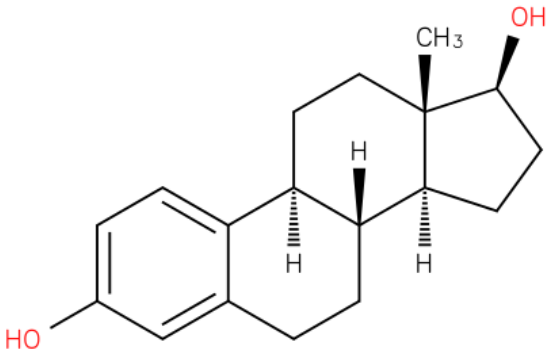

Fig. 1.2: Structure of E2 obtained from ("17-β Estradiol," 2015)

The E2 is produced mainly in the ovaries but it is also produced in the testicles too but in extremely low concentrations (Najafi et al., 2012). This hormone does not only affect the tissues in the reproductive system but other tissues such as bone, fat, skin, liver, and the brain (Najafi et al., 2012; Ryan, 1982). The E2 is not only found in humans and other mammals but it is also found in most vertebrates and crustaceans, insects, fish, and other animal species (Ruan, Zong, Zhao, Liu, & Chen, 2014). Besides the ovaries and testicles, E2 can also be produced in other tissues such as the adrenal glands, fat, liver, the breasts, and the brain (Sneader, 2006). Cholesterol is the main source of E2 by a series of different reactions resulting in different intermediates. Usually androstenedione is formed from cholesterol then changed into estrone by aromatase which is eventually converted into E2 or testosterone can be formed through the process of aromatization of androstenedione which is ultimately converted to E2 (Zhang and Zhou 2005). When women reach menopause, the production of E2 in the ovaries ceases or decrease to extremely low amounts but it is still needed in the body, for this reason E2 is produced synthetically for menopausal hormone therapy (Nokelainen 2000). 17-beta estradiol can also be used for treatment of hormone-sensitive cancers e.g. prostate cancer and regulation of estrones and menstrual cycle. For hormonal therapy, women with E2 deficiencies are commonly administered with it orally but not all of it is consumed in the body. Some of it is released from the body by urination and can find its way to the rivers, and wastewaters (Musgrave and Tattersall 2011). This hormone has been attracting a lot of attention as an environmental pollutant since it is a persistent organic pollutant and it is not easily broken down (Wu, Fang, Li, & Cui, 2014). Too much consumption of E2 can cause various problems both in humans and the environment such as breast cancer (Russo et al., 2006), infertility,

endometrial cancer, hormone imbalance, lung cancer, gynaecological problems etc. Environmentally it inhibits anaerobic microorganism activity, promotes generation and release of methane in water, promotes global warming and disturbs aquatic life (Ruan et al., 2014).

1.2 PROBLEM STATEMENT

There are several problems associated with conventional wastewater treatment techniques that are used all over the world. The first problem is that they fail to detect, extract, separate and remove most pharmaceutical pollutants. So these pollutants accumulate at the treatment plant and as a result the drinking water contains more pharmaceuticals than waste water itself (Murdoch, 2015). The second problem is that for those techniques that can detect and remove some pharmaceuticals, they cannot remove all of them. These are mostly limited by factors such as solids retention time (SRT) which may slow down the rate of treatment. If the SRT is maintained, it may lead to promoting the growth of biological community which is more diverse in nature but is probably able to degrade some of the pharmaceutical pollutants more effectively (Al-Farsi, Ahmed, Al-Busaidi, & Choudri, 2017; Dalkmann et al., 2012).
Some of these techniques are able to remove pharmaceuticals that are more hydrophobic and recalcitrant, but these pollutants are normally removed from the aqueous phase but accumulate in the Biosolids (Miao et al., 2005). Some remove the pollutants by means of converting them to other compounds through biological processes and oxidation. This may change the structural conformation but do not completely remove them (Kanakaraju, Glass, & Oelgemöller, 2018; Ternes et al., 2002).

Most conventional wastewater treatment methods are focused on the removal of the most common organic pollutants such as pathogens. Several studies have been conducted and show that pharmaceutical residues are present in both treated water and untreated water from wastewater treatment plants (Onesios, Yu, & Bouwer, 2009). Some pharmaceutical pollutants have proven to be resistant to removal when primary sedimentation and secondary microbial treatment processes are used. To serve as an example carbamazepine is not removed by secondary wastewater treatment and this pharmaceutical has even been detected at elevated concentrations in post-treated water than pre-treated water (Murdoch, 2015; Vieno, Tuhkanen, & Kronberg, 2007). Researchers believe that this increase in concentration may be attributed to metabolites of CBZ that are converted to CBZ during the process of microbial treatment (Murdoch, 2015). Other pharmaceutical drugs include the synthetic steroid estrogens which are also resistant to elimination by enhanced primary wastewater treatment and this is a problem

because they are found to accumulate in sewage sludge (Braga, Smythe, Schäfer, & Feitz, 2005).

Most researches are based on the investigation of the detection and removal of the parent pharmaceutical residue during the treatment process. So, these methods do not detect derivatives or metabolites present. Hence, the absence of the parent material in wastewater does not eliminate the presence of metabolites or derivatives. In addition, the pollutant might not be completely degraded but could have been transformed to another chemical due to chemical reactions that occur in the plant during the treatment process. This resultant chemical may remain undetected by the analytical methods that are being used (Onesios et al., 2009). It is vital that techniques for removal of pharmaceutical residues take into consideration the conversion parent pharmaceutical compounds to other compounds because of metabolism in the administrator, the action of microbial enzymes during wastewater treatment or by chemical or even light-induced conversions. Hence most researches now are focused on modifying the current methods in order to accommodate such transformations (Murdoch, 2015).

The most commonly used analytical techniques for the determination of the pharmaceutical pollutants are based on high performance liquid chromatography, gas chromatography both coupled with different detectors (Santana, Ferrera, Padrón, & Rodríguez, 2009). Since these pollutants are incorporated in complex matrices or are present ultra-trace levels, the above-mentioned analytical techniques are normally combined with sample preparation techniques such as solid phase extraction (SPE), solid phase microextraction (SPME), dispersive solid phase microextraction (DSPME) and liquid phase microextraction (LPME) that increases the detection limits of these techniques. Among these sample pre-treatment techniques, dispersive solid-phase extraction and solid phase microextraction are mostly used for preconcentration and clean up in the analysis of environmental samples. This is due to its attractive features such simplicity, rapidness and little consumption of organic solvents, safety and affordability that the possess (Lombardi, 2015).

1.3 JUSTIFICATION

Since the establishment of the problem of main conventional methods not being able to pick up, isolate and remove some metabolites, pharmaceuticals and some emerging pollutants, several techniques have been developed not only for this but also to simplify, reduce energy and time consumption at the same time being environmentally friendly. These techniques

include liquid-liquid extraction (LLE) (Islas et al., 2017), solid phase extraction (SPE) (Zhao et al., 2016), solid phase microextraction (SPME) (Kamalabadi, Mohammadi, & Alizadeh, 2016), microwave-assisted extraction (MAE) (Speltini et al., 2015), and pressurised liquid extraction (PLE) (Hoff, Pizzolato, Peralba, Díaz-Cruz, & Barceló, 2015). Although these techniques may, have enormous number of advantages, most of them require additional steps such as pre-treatment or some components needs to be manipulated depending on the analytes, and this consumes time as well as eliminates ease of use. The parent method used for extraction and clean up for analysing pharmaceuticals is SPE. Modification of this method has allowed the development of other methods such as DSPME, this technique allows for direct contact between sample and the sorbent and this is made possible dispersion by ultra-sonication this increases the number of sample particles that interact with the sorbent (Anumol et al., 2017; Lehotay, 2011). After dispersion the sorbent carrying the analytes is isolated mechanically by filtration, centrifugation or in the case of dispersive magnetic solid phase extraction (MDSPME), the analytes are separated by a magnet. After the solid phase is successfully separated, the analytes that are retained on to the sorbent can then be easily eluted by adding an organic eluent such as acetonitrile (Islas et al., 2017; Jamali, Firouzjah, & Rahnama, 2013; Posyniak, Zmudzki, & Mitrowska, 2005). Dispersion allows this technique to save time; this means more samples can be analysed adding to its list of advantages which include ease of use, versatility, and easy handling as compared to conventional techniques (Han, Sapozhnikova, & Lehotay, 2014)

DSPME was invented around the year 2000 and it has been used for variety applications throughout the world (Anastassiades, Lehotay, & Schenck, 2003). These varieties of applications include preconcentration, isolation, and cleaning in multiple analytical industries. The main advantages of this technique is that it forms part of the so-called quick, easy, cheap, effective, rugged, and safe quenchers (Naing, Li, & Lee, 2015). DSPE simplifies SPE as a clean-up technique by allowing more samples to be analysed in a very short period. This technique is much quicker and consumes very little amount of the solvent. A solid sorbent which is usually silica, carbon or polymer based is dispersed directly into the sample solution (Anumol et al., 2017; Han et al., 2014; Lehotay, 2011). More surface area of the sorbent is exposed to the analytes for retention by dispersion. Since its inception, DSPME has been widely accepted as an extraction and clean-up technique due to its flexibility, ease of use, quickness, good recovery, robustness, low solvent consumption, and the ability to combine with multiple detection techniques (Islas et al., 2017).

DSPME has been combined with multiple instrumental techniques for the detection and quantification of pharmaceutical residues in water, food and blood samples. These techniques include capillary electrophoresis coupled with diode array detection (Ibarra et al., 2011; Ibarra et al., 2012), ultraviolet-visible light detection (Hu et al. 2012), mass spectrometry (Domínguez-Álvarez et al., 2013), high performance liquid chromatography coupled with ultraviolet-visible light detection (Reyes-Gallardo et al., 2016), fluorescence (Hu et al., 2011), diode-array detection (Wang et al., 2016) and liquid chromatography coupled with mass spectrometry (Gao et al., 2010) to name but few. For the purpose of this work, DSPME and DMSPME are used coupled with HPLC that is coupled with DAD as a detector.

1.4 HYPOTHESIS

Organic pollutants of interest were found in wastewater. The organic pollutants were successfully adsorbed on to the surface of the adsorbent and released through desorption by an organic solvent. The amount of the organic pollutants desorbed were quantified using HPLC.

1.5 AIM AND OBJECTIVES

1.5.1 Main objective

The aim of the study is to synthesise and characterise nanometer-sized metal oxides coated carbon nanofibers then use them as adsorbents for extraction/preconcentration of selected organic pollutants in environmental samples.

1.5.2 Specific objectives

1. Preparation and characterisation of MgO-ZnO and Al_2O_3-Fe_3O_4 coated carbon nanofibers (MgO-ZnO@CNFs and Al_2O_3-Fe_3O_4@CNFs). To characterise the prepared nanomaterials, Fourier Transform Infrared Spectroscopy (FTIR), X-Ray diffraction (XRD), scanning electron microscope/ energy-dispersive spectroscopy (SEM/EDS), transmission electron microscope (TEM) and Brunauer–Emmett–Teller (BET) are used.
2. Development of dispersive solid phase microextraction based on MgO-ZnO@CNFs as an adsorbent for extraction and preconcetration of CBZ in wastewater and surface water. The factors affecting the preconcentration system were optimised using central composite design.

3. Application of Al_2O_3-Fe_3O_4@CNFs as an adsorbent for magnetic solid phase extraction of 17-β-estradiol in river water samples. Box-Behnken design was used for optimisation of the extraction method.

1.6 MINOR DISSERTATION SET UP

- **Chapter 1**: This chapter consist of the background information about water pollution in general then the impact of pharmaceuticals on pollution as well as some challenges encountered in removing them from water systems. Finally, the problem statement, justification of the study, aims and objectives are presented in this chapter.
- **Chapter 2**: This chapter focuses on the details about the materials used, the methodologies employed, the instrumentations as well as the optimisation techniques.
- **Chapter 3**: The focus of this chapter is on 17 β-estradiol, how it is used, produced and how it pollutes the environment as well as how to extract it and preconcentrated it from wastewater using UA-DMSPME as a sample treatment technique.
- **Chapter 4**: The focus of this chapter is on Carbamazepine, how it is used, produced and how it pollutes the environment and how it is extracted and preconcentrated from wastewater using UA-DSPME as a sample treatment technique.
- **Chapter 5**: In this chapter, all the results are summarised and the overall performance of the sample preparation methods and the adsorbent materials are laid out.

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CHAPTER 2:
LITERATURE REVIEW

2.1 INTRODUCTION

This chapter focuses on the literature review about the sample preparation techniques for the extraction and preconcentration of organic pollutants from environmental samples. These sample preparation techniques include ultrasound assisted dispersive solid phase microextraction and ultrasound assisted dispersive magnetic solid phase microextraction. The materials that are used as adsorbents in the extraction as well as preconcentration procedures and the characterization techniques are also discussed in this chapter. Overviews of the optimisation techniques that include multivariate tools are also discussed.

2.2 NANOMATERIALS BASED ADSORBENTS

Nanomaterials are defined as materials that have particle sizes that range from 1-100 nm (X-Z. Hua et al., 2012). These materials vary from their bulk materials because their properties change as their sizes change. This allows for manipulation of their characteristics by changing the size, shape and other properties which enables them to be used in a variety of different fields (Patzke, Krumeich, & Nesper, 2002). For this reason, nanomaterials have been used as adsorbents in adsorption processes especially in environmental remediation application (Singh, Barick, & Bahadur, 2011; Wang et al., 2012; Shen et al., 2009). This is because they have extremely large surface areas as compared to their bulk material, large number of pores, more exposed pores due to high surface area, this leads to high adsorption efficiencies and faster adsorption kinetics (Dil, Ghaedi, & Asfaram, 2017; Sadegh, Shahryari, & Masjedi, 2016; Theron, Walker, & Cloete, 2008). Additional advantages of these materials include easily functionalised, cost effective, eco-friendly, abundance (most are naturally occurring e.g. carbon). As a result, nanomaterials have attracted a lot of application in water treatment because they are the closest to meeting the ideal wastewater treatment requirements as adsorbents (Gupta et al. 2016). According to (Machida, Mochimaru, & Tatsumoto, 2006, Sharma et al., 2009 & Ali, 2012), an ideal adsorbent for wastewater treatment should fulfil the following requirements:

(i) Eco-friendly;

(ii) Exhibit a high adsorption capability and high selectivity towards target pollutants in aqueous media even at trace level concentrations;

(iii) The adsorbed pollutants be readily desorbed from the surface of the adsorbent, and

(iv) Reusable.

The most commonly used adsorbents include clay minerals, metal oxides, silica gel, zeolites, carbon nanomaterials, maize bran, activated carbon, organic polymers and activated alumina, among others (Gupta et al. 2015). Carbon based nanomaterials are the closest to meeting the above criteria and hence have been extensively used recent decades due to their nontoxicity and high adsorption capacities (Mauter & Elimelech, 2008). For example, activated carbon is one of the firstly used carbon-based adsorbents. However, the use of this material is limited to parts per million concentration levels in heavy metal extraction as it fails to remove them at parts per billion levels (Sadegh et al., 2017). As the development of nanotechnology continued, other materials were discovered to be better adsorbents than activated carbon. These materials include graphene oxide, carbon nanotubes, carbon nanofibers and fullerene (Wang 2012).

Metal oxide nanoparticles are other inorganic nanomaterials that are commonly used in adsorption application, especially for wastewater treatment (Vinod Kumar Gupta et al., 2015). This is due to their unique properties that include high surface area, selectivity, fast kinetics and easy functionalisation which can enhance specific characteristics by adding other materials (Gupta et al., 2015). Moreover, metal oxides are environmentally friendly, this is due to their low solubility which helps minimise secondary pollution (Gupta et al. 2015). These metal oxides include zinc oxide (Dimapilis, Hsu, Mendoza, & Lu, 2018), titanium oxide (Luo et al., 2010), magnesium oxide (Schiller, Tallman, & Khalafalla, 1984), ferric oxide (Feng et al. 2012), aluminium oxide (Lee, Wang, Guo, Hu, & Ong, 2015), cerium oxide (Cao et al., 2010) and copper oxide (Goswami, Raul, & Purkait, 2012), manganese oxide (Gupta et al. 2011) to name a few. Combining the properties of metal oxides and carbon based adsorbents, scientist came up with nanocomposites which are resultant materials containing all the desirable properties from both materials (Nabiyouni et al., 2014). This is also used in waste wastewater treatment as it brings advantages such as easy recovery, increases adsorption speed, increases

surface area, increases number of pores, increases selectivity, decreases solubility (Nabiyouni et al., 2014). Hence, in this work carbon nanofiber-metal oxide nanocomposites were utilised for extraction and preconcentration of selected emerging contaminants from aqueous media.

2.2.1 Carbon nanofibers

Carbon nanofibers (CNFs) are classified as sp2-based linear filaments that have very large surface area and an average diameter of 100 nm. The structure of carbon nanofibers is what gives it its exceptional properties and unlimited applicability (Kaerkitcha, Chuangchote, & Sagawa, 2016). It is linear, sp2-based with 1 double bond and 2 single bonded discontinuous filaments, where the aspect ratio is greater than 100 (Kim, Hayashi, Endo, & Dresselhaus, 2013). It contains stacked layers of graphene that are stacked perpendicular to the fiber axis. The stacking is in a form of herringbone/cup-stacking which means the graphene layers are stacked at an angle between parallel and perpendicular to the fiber axis (Park, Engel, Crowe, Gilbert, & Rodriguez, 2000). The layering arrangements are made possible by the growth mechanism of the CNFs that depends on the geometric facets of a metallic catalyst particle and the gaseous carbon feedstock (hydrocarbon or CO gas) that is introduced during CNFs processing (Lichao Feng, Xie, & Zhong, 2014). Such general classifications leave further room for additional categories of carbon-based nanoscale fibers (Poveda & Gupta, 2016). There are mainly three types of CNFs which are classified according to their shapes:

(i) the herringbone, in which the graphene layers are stacked obliquely with respect to the fiber axis;
(ii) the platelet, in which the graphene layers are perpendicular to the fiber axis; and
(iii) the ribbon, in which the graphene layers are parallel to the growth axis (Naing, Li, & Lee, 2015).

Different structures of CNFs provide different properties such as superior mechanical strength, low grain boundary, 1-D structure with high alignment and high surface area to volume ratio (Dhineshbabu, Karunakaran, Suriyaprabha, Manivasakan, & Rajendran, 2014). Therefore, because of their unique properties, the CNFs are interesting materials for various applications such as selective adsorption, hydrogen storage, polymer reinforcement and catalysts support, photocatalysis (Kim, 2014; Mu, 2011) electrodes for supercapacitors (Tran, 2013; Xu, 2015), rechargeable lithium-ion batteries (Chen, 2012; Han et al., 2012; Liu, 2011;

Wu, 2014), photovoltaic cells (Park, 2013; Sebastián, 2014) capacitive deionization process (El-Deen, 2014) and selective screening (Wang, 2015). Moreover, their properties can be improved by appropriate selection of precursor material, production process, and treatment. Physical properties of the obtained nanofibers are mostly dictated by their morphology, fiber diameter, and specific surface area (Kaerkitcha et al., 2016).

2.2.2 Nanometer sized metal oxides

2.2.2.1 Nanometer sized aluminium oxide (Nano-Al_2O_3)

Aluminium oxide occurs naturally in the mineral bauxite that is mined all over the world and nanosized aluminium oxide can be manufactured synthetically by means of the Bayer method. This can be done by crushing, drying, and dissolving bauxite in a concentrated sodium hydroxide solution. From this, nano-sized Al_2O_3 can then be obtained (US Research Nanomaterials, 2018). Nano-Al_2O_3 are nanoparticles with sizes in the nanometer range with high catalytic activity and low melting temperatures (Koli, Agnihotri, & Purohit, 2014). They are chemically and thermally very stable and are almost insoluble in water, acids and bases. They have very high surface area and high defectiveness (US Research Nanomaterials, 2018). These characteristics enable them to be applied in various fields such as engineering and medicines like aerospace, defence, automobiles, electronics, materials, chemistry, energy, environmental sciences, information & communication, consumer goods and biotechnology (Koli et al., 2014). Due to their thermal stability, aluminium oxide nanoparticles are used as catalyst carriers and adsorbents in the petroleum and chemical industries (Koli et al., 2014). Sintered into porous structures and applied to coarser substrates, nanoscale aluminium oxide can also be used for nanofiltration (Vinod Kumar Gupta et al., 2015). In this work nanometer, sized Al_2O_3 is mixed with other metal oxide and carbon nanofibers to form a composite. The composite will have much greater advantages in extraction of organic pollutants since nano-sized aluminium oxide can be used as adsorbent material.

2.2.2.2 Magnetite (Fe_3O_4)

Magnetite (Fe_3O_4) is a naturally occurring mineral that is one of the main iron ores. This mineral is ferrimagnetic which means it is attracted to magnets and by magnetising it, it can be

a permanent magnet (Wasilewski, Peter; Kletetschka, 1999). It is the most magnetic of all the naturally occurring minerals on earth. This naturally occurring magnetic compound clearly contains many interesting properties and potential for various applications (Goya et al., 2003). Compared to magnetite particles of micro-scaled, nano-scaled magnetite particles are about a million times smaller by volume and exhibit properties that are much different (Mahdavi et al., 2012). The sizes of these particles make it extremely difficult to synthesise them, but recently a couple of methods have been developed by different scientists making it easier to produce them (Ye et al., 2014). Generally, there are two main methods of synthesising magnetite nano particles; thermochemical precipitation reactions in solution, or the chemical reactions in specialized reactors (Mahdavi et al., 2012). The chemical reaction methods include: solution combustion, co-precipitation, sol-gel, emulsion technique, hydrothermal preparation (Mahdavi et al., 2012).

In this study magnetite was synthesised using coprecipitation method which is regarded as an efficient way to produce of ultrafine and mono-dispersed magnetic nano particles (Nabiyouni et al., 2014). Magnetite nanoparticles have received well-deserved attention due to their interesting properties such as their great biocompatibility, high chemical stability, super paramagnetic properties, non-toxicity and easy synthesis process (Herrero-Latorre et al, 2015). These properties attracted much attention in various fields of magnetic recording media medical applications and recovery of hazardous wastes (Mahdavi et al., 2012). Most of the unique magnetic properties of magnetite are due to the transfer of electrons between Fe^{3+} and Fe^{2+} in the octahedral sites (Nabiyouni et al., 2014). In this work, magnetite is bonded with other oxides as well as carbon nanofibers for the extraction of various organic pollutants from environmental samples. Its superparamagnetism characteristic is used to facilitates magnetic separation after the extraction (Zhang et al., 2010).

2.2.2.3 Zinc oxide (ZnO)

Zinc oxide (ZnO) is an inorganic compound that is white in colour and mostly found in powder form. This compound has been used for centuries mainly as an additive in numerous materials and products including rubbers, plastics, ceramics, glass, cement, lubricants, paints, ointments, adhesives to name but few (Hernández Battez et al., 2008). The fundamental characteristic of ZnO is its band gap energy which is 3.37 eV at room temperature; this energy provides zinc

oxide the incredible properties such as its catalytic, electrical, optoelectronic, and photochemical properties (Wang, 2004). ZnO nanoparticles have received massive attention in various industries due to their excellent performance in electronics, optics and photonics (Wang, 2004). Besides the band gap energy, reducing the size of ZnO particles to nano size introduces new properties such as electrical, mechanical, chemical and optical properties which are largely believed to be the result of surface and quantum confinement effects (Koli et al., 2014). ZnO nanoparticles have a diverse group of growth morphologies, such as nanocombs, nanorings, nanohelixes/nanosprings, nanobelts, nanowires and nanocages (Wang, 2004). ZnO nanoparticles have large surface area which comes as a great advantage in the catalysis industry; large surface area increases the material's catalytic activity (Amendola et al., 2014). Different morphologies of ZnO nanoparticles requires different methods, these methods determine the physical and chemical properties of such morphologies (Amendola et al., 2014). When ZnO nanoparticles are combined with carbon based materials such as carbon nano tubes, activated carbon, graphene or carbon nanofibers, they provides synergistic effects that are advantageous for different extraction applications (Wang, 2004). Due to molecular sieve effects, ZnO nanoparticles can effectively extract target compounds and that is why it has been used over different support materials in preconcentration (Zhang et al., 2016).

2.2.2.4 Magnesium oxide (MgO)

Magnesium oxide (MgO) is an inorganic compound that occurs naturally as the mineral periclase. When in aqueous medium, it reacts fast with the water to form magnesium hydroxide (PubChem Compound Database, 2018). Magnesium oxide nanoparticles are odourless and non-toxic and they possess high hardness, high purity and a high melting point (Schiller et al., 1984). Magnesium oxide nanoparticles have multiple properties that enable them to be applied in various fields such as in electronics, catalysis, ceramics, petrochemical products, coatings and many other fields (Zhang et al., 2007). Magnesium oxide (MgO) nanostructures have drawn special attention because of their important applications in the areas of catalysis, refractory materials, and superconductors (Karimi et al., 2015). An important aspect to consider when dealing with MgO nanoparticles is the possible presence of oxygen vacancies. The latter can have a tremendous influence on the electronic and chemical properties of the nanoparticles (Lawrence, 2015). MgO displays unique selectivity for the separation of basic compounds in

normal-phase liquid chromatography (Zhang et al. 2007), this prompted scientists to investigate its selectivity in solid phase extraction. The high adsorption capacity for electron-rich compounds is attributed to interactions between oxygen vacancies at the surface or sub-surface of MgO and lone pairs of electrons on the analyte (Zhang et al. 2007).

Theoretical studies have shown that the presence of oxygen vacancies is essential for having magnesium oxide nanoparticles with high chemical activity. In fact, the O vacancies are so reactive that they may not be stable under the chemical environment of most catalytic reactions (Zhang et al., 2007). For several industrial applications MgO is mixed with small amounts of a transition metals, or another metal oxide (AZoNano, 2013). This can induce structural transformations and be used to stabilise MgO nanoparticles that expose (110) or (111) faces (Karimi et al., 2015). The doped MgO nanoparticles show high surface reactivity, and high chemical and thermal stability, which makes MgO a promising material for applications in extraction (Lawrence, 2015).

2.2.3 Nanocomposites based adsorbents

2.2.3.1 ZnO-MgO@CNFs nanocomposite

ZnO–MgO nanocomposite exhibit enhanced optical properties that results partly from the different crystallites or electronic coupling between ZnO and MgO which enhances the band gap (Mihai et al., 2010). However, they are unstable in acidic conditions due to aggregation and they show poor dispersivity, low specific area and all these factors can lower their activity in extraction processes (Mihai et al., 2010). However, in order to eliminate these challenges, several scientists have been trying to produce ZnO-MgO in composite or alloy forms by appending or binding ZnO-MgO into a stable inorganic support such as silica, graphene, carbon nanotubes, carbon nanofiber, MCM-41, and zeolites (Karimi et al., 2015). In this work, ZnO-MgO@CNFs nanocomposite is prepared in order to extract organic pollutants from environmental water samples. Combining the selective adsorption properties of carbon nanofibers, the molecular sieve effects of ZnO nanoparticles, unique selectivity for the separation and high adsorption capacity of MgO gives this composite the best chance of extraction not just organic but all basic pollutants (Karimi et al., 2015). The novelty of this composite is intriguing, because it combines the best feature of each component in the

composite and can be a great turning point in water treatment as it can also be used for water purification (Sadegh et al., 2017). The depth of this composite lie in the characteristics of the carbon nanofibers as they are able to bond with various metal oxides without their own extraction properties being jeopardised but instead they become enhanced (Karimi et al., 2015). Due to their high surface area per volume, they can accommodate the metal oxide particles in larger numbers and their unique morphologies enhances the adsorption capacity (Kaerkitcha et al., 2016).

2.2.3.2 Al_2O_3-Fe_3O_4@CNFs nanocomposite

Carbon materials have been widely used for the preconcentration of environmental organic pollutants because of their favourable separation ability, excellent stability and long lifetime (Karimi et al., 2015). Although carbon nanofibers have not been used as much as other carbon support such as activated carbon, carbon nanotubes and graphene, they have properties that such as high adsorption capacity, high surface area and high selectivity which are very important in extraction processes. Therefore, carbon nanofibers are expected to perform excellently in the aspects of adsorption and extraction of the organic pollutants from water sample (Chen & Tang, 2007). The extraction properties of carbon based materials including carbon nanofiber can be enhanced by addition of various metal oxides; this has been reported in literature (Djozan, Assadi, & Haddadi, 2001). Among various nanometer-sized metal oxides the iron and aluminium oxide nanoparticles have been widely used due to their superior advantages such as low cost, extensive availability, thermal stability and remarkable adsorption capacity (Djozan et al., 2001). These advantages multiply when magnetite is introduced. It has been reported that Fe_3O_4-Al_2O_3 shows excellent qualities such as high sensitivity, low cost, high sample throughput, relatively low detection limits, high enrichment factors as well as high precision and accuracy (Djozan et al., 2001; Nyaba, Matong, & Nomngongo, 2016). Due to high adsorption capacity and potential reusability, Fe_3O_4-Al_2O_3 nanocomposite can be paired with carbon nanofibers and used as an effective adsorbent in environmental water treatment for preconcentration of organic pollutants (Nyaba et al., 2016).

2.3 SOLID PHASE BASED SAMPLE PREPARATION METHODS

2.3.1 Dispersive solid phase microextraction

Solid phase microextraction (SPME) is an innovative, solvent-free sample preparation method, it is a relatively new extraction technique that was first introduced by Pawliszyn and his co-workers early in the last decade of the 20th century (Djozan et al., 2001). This technique is unique as it is fast, affordable, and versatile. So given these advantages, SPME has attracted a lot of attention in the past decade as it can be applied as an alternative to most of the conventional sampling techniques so it has been widely accepted in many fields of science throughout the world (Islas, Ibarra, Hernandez, Miranda, & Cepeda, 2017). Recently, it has been applied in various fields of science because it is quick, easy, cheap, effective, rugged, and safe approach for extraction of pesticides, organic pollutants, heavy metals etc. (Cárdenas Aranzana, 2010). Other advantages of this method include ease of use, rapidness, high sensitivity, safe sampling, extraction, concentration, and it simplifies sample introduction procedures in GC and HPLC into a single step (Djozan et al., 2001). In this method, sorbent should be highly selective and preferably interact with the analyte of interest but not the endogenous matrix components.

2.3.2 Magnetic solid phase microextraction

Magnetic solid-phase microextraction (MSPME) is a new mode of SPE based on the use of magnetic or magnetizable adsorbents. MSPME is a modern and miniaturised technique that demands small volume of sample and solvents for extraction and desorption. Furthermore, this technique allows the concentration of the analytes, increasing method detectability (Herrero-Latorre et al., 2015). In addition, the high contact surface provided by dispersing the sorbent into the matrix using ultrasound, yields a high recovery of the analytes (Vasconcelos & Fernandes, 2017). The adsorbent does not need to be packed into the SPE cartridge; instead, it can be dispersed in a sample solution or suspension. The powdery magnetic adsorbent can be reversibly agglomerated and re-dispersed in solution or suspensions by the applying and removing a suitable magnetic field. Thus, the phase separation could be conveniently conducted. From the view of mass transfer, the MSPE mode can also facilitate mass transfer of analytes by drastically increasing the interfacial area between the solid adsorbent and sample

solution (Yan Liu, Li, & Lin, 2009). The magnetic sorbent which in this work is Al_2O_3-Fe_3O_4@CNFs is added to the sample solution and the target analytes are adsorbed onto the surface of the magnetic sorbent particles. Analyte- Al_2O_3-Fe_3O_4@CNFs are separated from the sample solution by application of an external magnetic field and, after elution with the appropriate solvent, the recovered analytes are analysed by HPLC. This approach has several advantages over conventional solid phase extraction technique as it is not time consuming and tedious on-column SPE procedures and it provides a rapid and easy analyte separation that avoids the need for centrifugation or filtration steps (Vasconcelos & Fernandes, 2017). Therefore, in the past few years a great deal of research has been focused on MSPE, including the development of new sorbents and novel automation strategies. In recent years, the use of magnetic carbon nanotubes (MCNTs) as a sorption substrate in MSPME has become an active area of research (Herrero-Latorre et al., 2015). These materials have exceptional mechanical, electrical, optical and magnetic properties and they also have an extremely large surface area and varied possibilities for functionalisation (Herrero-Latorre et al., 2015). Carbon nanofibers are very similar to carbon nano tubes as they also have exceptional properties such as high selectivity, sensitivity mechanical properties; these are also enhanced by addition of the Al_2O_3 and Fe_3O_4 metal oxides.

2.4 OPTIMISATION METHODOLOGIES

2.4.1 Univariate or one factor at a time (OFAT)

Univariate or one factor at a time (OFAT) is a traditional experimental design method in which one factor is tested while others remain in a fixed position. After the study of one factor is complete and fully understood, the other factors are studied one at a time. This is carried on until all the factors affecting the experiment are fully studied and characterised (Figardf, Sirhan, & Tan, 2015). This method has been in existence for centuries and many developments have been done to improve it. Hence, in most cases OFAT is used by non-experts or in experiments with affordable data that is available in abundance. This experimental design requires little to no statistical knowledge for its application in the execution of data analysis. OFAT is still utilised by many organisations around the world for the determination of the conditions of the main factor (Wahid & Nadir, 2013). The first drawback of this experimental design is the low

precision of estimated effects of the factor in question due to experimental conclusions that are determined after data collection of each trial run by comparison of the obtained outcome with the previous results (Wahid & Nadir, 2013). Typically, the effect of each factor is estimated by only two observations in OFAT experiments. Hence this type of experiments are regarded as trial and error which require luck or intuition to be successful (Wahid & Nadir, 2013). Another disadvantage of this design is that if the best setting of a factor(s) requires interaction with other factors, it may be missed (Figard, Laboratories, & Park, 2009). Due to such problems, OFAT can prove to be inefficient and unreliable. This can lead to inaccurate or even wrong optimum conditions that can ruin the experiment. This approach is time consuming, especially with large amounts of data and various factors to be analysed (Wahid & Nadir, 2013). Scientist came up with other experimental designs to obtain optimum conditions. These designs include multivariate optimisation which vary several factors at the same time and have proven to be more efficient when analysing two or more factors (Figard, Sirhan, and Tan 2015).

2.4.2 Multivariate optimisation

Multivariate optimisation is a type of experimental design that aims to achieve optimum conditions by optimising several factors simultaneously (Jones, 2001). In this type of experimental design, there are two types of variables, namely: the response and the factor. The responses are regarded as the dependent variables since their results or outcome depend on the levels of the factors, these may be classified as qualitative or quantitative (Shahmohammadi et al., 2016). All possible combinations of all factors are included at all levels in this type of experimental design. More than two levels can be investigated but this also has an impact on the experiments conducted. For example if there are two factors at x levels then 2x experiments are required for a full factorial design (Jones, 2001). Varying different factors and responses at different levels allows for designed experiments that are more effective in determining the effect of two or more factors on a response than the OFAT experimental design. Multivariate design allows for less time, materials and number of experiments conducted while using fewer resources in order to find optimum conditions (Khan, Afzaal, & Imran, 2018). This is a major advantage in industries where experiments are conducted in large scale, time consuming and are very expensive. High precision of the estimates of the effects of each factor are reached in multivariate designs (Jones, 2001). The high precision is due to the use of more observations

in order to estimate an effect. With this method, the impact that results from interaction between factors can be investigated (Figard et al., 2009).

2.4.3 Box–Behnken design

Box-Behnken designs are a class of independent quadratic designs that are rotatable or nearly rotatable second-order designs based on three-level incomplete factorial designs (Leivisk, 2013). Treatments of combinations are at the midpoints of the edges of process space and centre in this type of design (Leivisk, 2013). For full analysis of each factor, three levels must be investigated. This has a limitation in capability for orthogonal blocking when compared with central composite design (Wahid & Nadir, 2013). In Box-Behnken experimental designs, two levels of factorial designs are combined with incomplete block designs that are arranged in a certain manner (Shahmohammadi et al., 2016). In cases with three or four factors, Box-Behnken designs require fewer treatment combinations than central composite design. To get the best out of this experimental design, combined factor extremes should be avoided; this also helps the missing corners to be useful. This way potential loss of data is prevented (Leivisk, 2013).

These designs help to limit the size of the sample when number of parameters increase. Sample size that is enough for the estimation of all the factors and their levels is investigated and kept constant in second degree least squares approximation of the polynomial (Figardf et al., 2015). In this design, a block of samples with a two-level factorial design is repeated over varying sets of parameters. All the important factors such as the size of the fractional and the number of block that are investigated all rely on the number of parameters and these are selected in order for the design to accurately approximate the criterion of rotatability (Cavazzuti, 2013). In order for an experimental design to be termed rotatable its variance of the predicted response at any point must be a function of the distance from the central point alone (Cavazzuti, 2013).

2.5 ANALYTICAL AND CHARACTERISATION TECHNIQUES

2.5.1 X-ray diffraction (XRD)

X-ray diffraction (XRD) is a technique that uses x-ray beams to cause an interference pattern of waves in crystalline atoms due to their uniform spacing. This technique is used widely in a many fields of studies for different purposes such as phase identification, verifying the crystallinity of materials, texture, the average sizes of particles, crystal defects and strain (Bunaciu, Udriştioiu, & Aboul-Enein, 2015). The way that this works in the instrument is that monochromatic beams of X-rays are scattered at specific angles set by their respective planes lattice which interfere with each other constructively to produce X-ray diffraction peaks (Riley, 1970). The intensities of these peaks are determined by the distribution of atoms in the lattice. Hence X-ray diffraction pattern fingerprint shows the arrangement of atoms in a sample (Bunaciu et al., 2015). Fig 2.1 shows the basic principle of the XRD instrument and its components. Typically, this instrument consists of three basic components: an X-ray tube the produces the incident radiation, a sample holder, and an X-ray detector for capturing, recording and identification (Connolly, 2007). The incident beam produces X-rays through the cathode ray tube and it is filtered in order to produce monochromatic radiation then they are aligned with the crystalline material and shot towards it. The sample then interacts with the incident beam as it passes through it to produce waves that interfere with each other constructively when Bragg's law is met. This law can be expressed as an equation as shown below:

$$n\lambda = 2d\sin\theta$$

Where n is a positive integer, λ is the wavelength of the incident beam, d is the distance between atomic layers in a crystal and θ is the angle at which the X-rays are diffracted.

The transmitted beam is diffracted at an angle then driven to the detector for recording. Since the powder is randomly orientated, a range of all possible diffraction patterns of the lattice is attained. By converting the diffracted peaks to the d-spacing allows for identification of compounds in the sample since every compound has its own set of distinctive d-spacing parameters (Bunaciu et al., 2015). The identification is made possible by comparison of the d-spacing values with the standard reference patterns (Bunaciu et al., 2015).

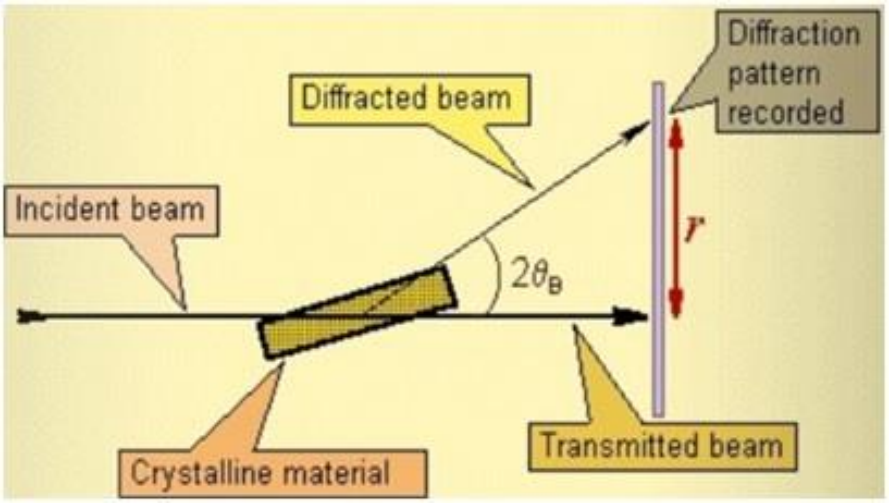

Fig. 2.1: Schematic representation of the XRD principle, adopted from: ("XRF vs XRD | Difference between XRF and XRD," 2016)

2.5.2 Transmission electron microscopy (TEM)

Transmission electron microscopy (TEM) can be defined as a type of microscopic technique that uses a beam of electrons accelerated at speeds close to that of the speed of light to be transmitted through an ultrathin specimen containing a sample (Saka, 1992). The beam of electrons interacts with the sample as it passes through the specimen to produce and image which is then magnified and focused onto processing device which can be the detector in a form of a camera (Zinin, 2010).

As seen in Fig. 2.2, TEM consist of four major components in its system, which are:

(i) the electron source composed of the power cable and the filament which is used to produce and fire the electrons towards the sample at very high speed,

(ii) the electromagnetic lens system containing the magnetic lens, electron beam and a vacuum pipe,

(iii) the sample holder consisting of the specimen and airlock and

(iv) finally the imaging system containing the projection lens and the imaging plate.

TEM operates under a vacuum and uses electromagnetic fields to focus and direct electron beams. Its high magnification is due to the short wavelengths of the electrons which are more than 8000 times smaller and are accelerated at voltages around 50 kV (Serrano, Silva, & Silva, 2010). The higher the accelerating voltage the shorter the wavelength and the higher the

resolving power. The electron source produces the electron then the electron gun fires them towards the sample at a specific voltage, when this beam of electron hits the sample, a number of interactions occur (Voutou, Stefanaki, & Giannakopoulos, 2008). This enables TEM to be versatile due to the large number of interactions that occur. These interactions include: transmission of electrons through the sample, generation of secondary electrons upon collision, backscattering of electrons, continuum x-rays, lattice vibrations, electrical current, generation of electron and hole paring. All these interactions can be used to extract information about the sample (Serrano et al., 2010; Steinbrecher, 2004). The high voltage gives the electrons large amounts of energy which enables TEM to examine objects at a very small scale which gives distinctive information (Voutou et al., 2008). This includes the morphology which consists of particle size, shape as well as distribution, these properties are extremely important because they can determine the behaviour of an object under various conditions (Bunaciu et al., 2015; Ruska, 1986). The composition of a material including the percentages of each element can also be evaluated using TEM coupled with energy dispersive spectroscopic (EDS) analysis. The interaction of compounds as well as incorporation of different materials on to each other can also be evaluated using TEM. This technique can also determine the crystallographic arrangement of atoms of a material (Boal et al., 2000; Post, 1990).

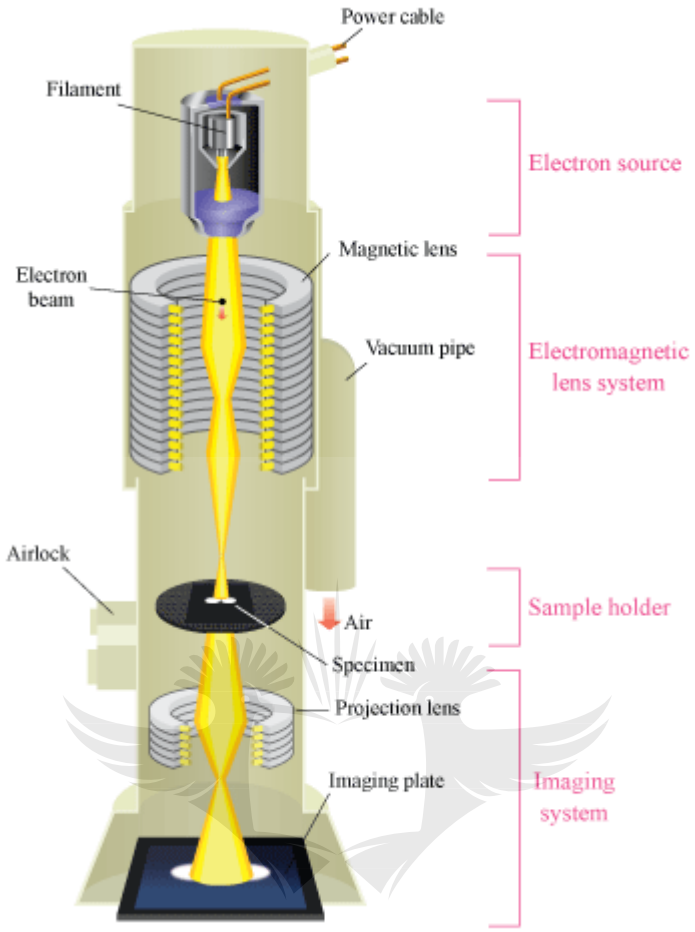

Fig. 2.2: Schematic representation of TEM principle adopted from (Ma, Shieh, & Qiao, 2006)

The unique advantages that TEM provides have led to its application in various fields of science. For example, it has been used in chemistry, biology, physics, medical sciences, engineering and geology. TEM has also been used in therombosis and atherosclerosis (Abela et al., 2003; Ma, Aziz, Huang, & Abela, 2006; Prieto et al., 2002). The general procedure for application of TEM are very simple and have been mostly used in chemistry, biology and medical sciences, these include sample collection, dispersion, stabilising, dehydration, deposition on ultrathin section usually in a form of a grid e.g. cupper grid, magnification and imaging (Ma, Shieh, et al., 2006).

2.5.3 Scanning Electron Microscope/Energy Dispersive Spectroscopy (SEM/EDS)

Scanning electron microscope (SEM) is an analytical technique in which a beam of electrons are used to scan a sample's surface therefore producing images (Srivastava, 2012). The interaction of this focused beam of electrons with the sample provides information about it such as elemental composition and the surface topography. The images are produced through the movement of the beam which is in a form of raster scanning (Voutou et al., 2008). The electrons from SEM are of high energy, up to 40 keV is produced by a fine probe then focussed onto the surface of the sample by magnetic fields to scan it in a series of parallel lines. Through this scanning a number of signals are generated as a result of the beam of electron's interaction with the sample's surface (Nabiyouni et al., 2014). These signals are sent to the detector in order to produce images and elemental composition. Secondary electrons are also produced through the interaction of the incident beam of electrons with the sample and have lower energies (Bogner, Jouneau, Thollet, Basset, & Gauthier, 2007).

A typical SEM instrument consists of five main components which include, the electron gun, condenser lens, scanning coil, the specimen stage and the detector (Kaech, 2002) (Fig. 2.3). The electron gun produces the electrons by means of thermionic heat. The electron gun applies thermal energy to the electrons in order to accelerate them from 0-40 kV depending on the type of instrument (M.-J. Park, Kim, Park, Jang, & Han, 2008). These electrons are condensed to form a narrow beam which is shot towards the specimen (Saka, 1992). The most common electron source is the tungsten filament due to its high melting point. This type of electron source consists of a V-shaped wire of tungsten that is upside down, it has a length of 100 µm and it is heated in order to produce electrons (Hafner, 2007).

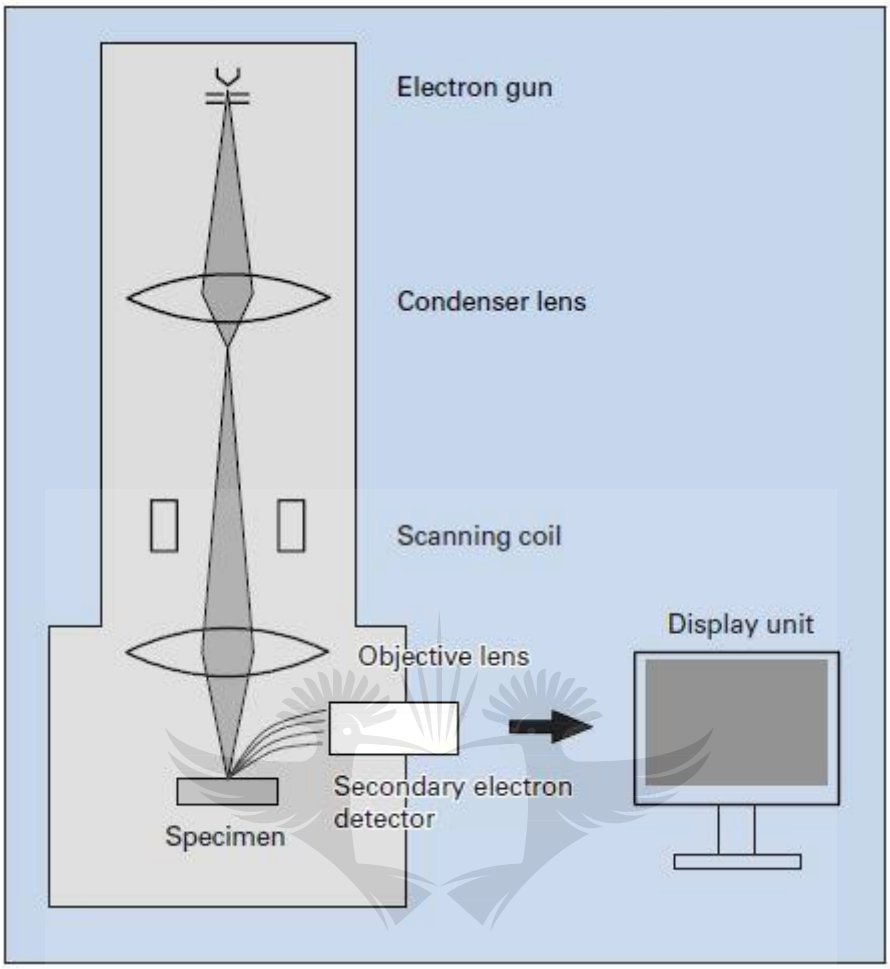

Fig. 2.3: Schematic representation of SEM principle adopted from (JEOL, 2006)

The beam of electrons is then accelerated down the column under vacuum. The vacuum conditions are there so as to prevent interaction of atoms or molecules available in the column with the beam, which eliminates interferences (Choudhary et al., 2017). The path followed by the beam of electrons is directed by the lenses and the condenser narrows the beam to a specified size, this in turn defines the resolution of the instrument. The primary function of the objective lens is to focus the accelerated beam of electron onto the specimen (Park et al., 2008). The lenses are made of magnets and hence they use magnetic fields in order to direct the path of the electron beam. A raster pattern is used to expose the beam onto the specimen by using the scanning coil (Park et al., 2008). The specimen stage or sample chamber is where the sample placed during the analysis. This is an extremely important component of the instrument because if the specimen is not placed correctly or the stage is not rigid, the analysis will fail.

Hence, the stage is made to be very sturdy and is protected against vibrations. Some instruments are even installed onto the ground to prevent vibrations. The function of sample chambers is not only to hold the sample and keep it still but they are also used to rotate the stage to change from one sample to the next, to place the sample in different angles and also to move it around so that its different sections can also be examined (Kaech, 2002).

As the electron beam hits the specimen, multiple interactions take place resulting in different types of electrons. Several detectors are required to analyse different types of electrons resulting from these interactions. These electrons include back-scattered electrons, x-rays and secondary electrons. Backscattered electrons are formed when electrons are emitted from the sample after interacting with the electron beam; hence, a backscattered electron detector is placed above the sample to detect them; these electrons can provide information about the topography of the sample. Secondary electrons are produced by atoms on the surface of the sample, these can only be detected if they have enough energy to leave the surface of the sample (Zavagli & Ricci, 1983). Hence a secondary electron detector also known as Everhart-Thornley detector is placed on the side of the sample chamber at a specific angle in order to detect them; these electrons can give surface information such as composition of the sample (Kaech, 2002). X-ray detectors are also used to give information about the composition of the sample through EDS. Images produced from the detectors can show contrast information in different sections of the sample with varying chemical make-up since elements with high atomic mass are brighter than those with low atomic mass (Zavagli & Ricci, 1983).

Scanning electron microscopy has many applications in various fields due to its growth in development, adaptability and high quality. This instrument can be used in material science for analysing nanotubes and nanofibers, nanocomposites semi and superconductors. SEM/EDS can detect and analyse fractures on the surface of a sample, give accurate information about the arrangement of microstructures, detect contaminations on the surface of the sample, give spatial variations in chemical compositions of the sample, validation of crystallinity of structures and quantitative analysis (Choudhary & Ka, 2017). SEM instruments can be used in a variety of science research laboratories such as chemistry, geology, biology, aerospace, medical, physics, metallurgy and forensic science (García-Veigas et al., 2012). The advantages that SEM instruments provide are that they can analyse a variety of different materials both at high and low magnification without sacrificing depth of focus hence they can be used almost

in every scientific lab. SEM's impressive properties such as the ability to provide three-dimensional and topographical images with great detail and the availability of multiple detectors which provide different types of information about the sample has attracted application in art. Digital artwork has been utilised from micrographs produced from SEM instruments. SEM/EDS is also used in forensic science in order to solve crimes since it can examine gunshot residues and determine its composition, analyse if bank notes are real or fake, paint analysis (Xiao & Gao, 2012). So many applications make SEM an essential tool in science.

2.5.4 Fourier transform infrared spectroscopy (FTIR)

Fourier transform infrared spectroscopy (FTIR) is a spectroscopic technique that uses infrared radiation to pass through a sample in order to obtain a spectrum (Hsieh, 2008). This spectrum can be used to collect high-spectral-resolution data over a wide spectral range which determines the functional groups of the sample (Hsieh, 2008). In typical FTIR spectrometer (Fig. 2.4), an infrared beam is passed through the sample chamber to interact with the sample and measured against a reference beam at each every wavelength to form a spectrum (Mecozzi et al., 2009). The beam-splitter plays a very significant role in FTIR spectrometry, as it is the heart of the interferometer. The beam-splitter is a half-silvered mirror that reflects only half of the incident beam while the other half is transmitted (Srivastava, 2012). One half of the beam goes to the moving mirror of the interferometer while the other goes to the stationary mirror of the interferometer (Srivastava, 2012). The two beams are then reflected back to the beam-splitter to generate an interferogram by the two mirrors then one is half reflected while the other is transmitted. This results in two beams given out in which one of them makes its way to the detector and the other to the source (Mecozzi et al., 2009). Since the movement of the two beams is different, this causes a difference in displacement of the moving mirror, so the interference patterns produced are different. These interference patterns are then detected and their variation in terms of energy is what is measured as the spectrum (Ramaiah & Bhatia, 2017).

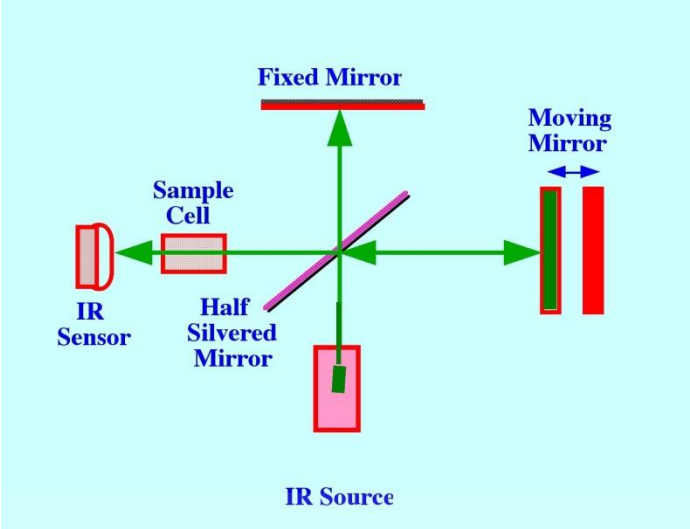

Fig. 2.4: Schematic representation of FTIR principle adopted from (Project8, 2018)

FTIR can be used to analyse sections of a sample to detect contaminants or functional groups that are not supposed to be there (Yongliang Liu & Kim, 2017). It can be used to analyse thin films and nanoscience to detect possible by-products interferences as well as purity of a material (Huth et al., 2012). It can be used in environmental sciences to tests the quality of the air water and soil (Mecozzi et al., 2009). Since it is environmentally friendly it can also be used to address the issues regarding environmental health concerns that are caused by the constant increase of pollution (Mecozzi et al., 2009).

2.5.5 Brunauer-Emmett-Teller (BET)

Brunauer-Emmett-Teller (BET) is an analytical technique named after the three scientists Stephen Brunauer, Paul Hugh Emmett, and Edward Teller who developed the mathematics behind its operation (Hwang & Barron, 2015). These scientists discovered a way of determining the specific surface area of a sample, micro-pore analysis, porosity and the pore size distribution all from adsorption and desorption of a gas (Brame & Griggs, 2016). This technique uses a gas that is chemically inert to the sample as a multilayer adsorption material to calculate the specific area (Fig. 2.5). The most widely used adsorbate material for probing is nitrogen gas. Because of this, most BET analysis are undertaken at -196.15 ℃ which is the boiling point of nitrogen (Sing, 2001). Additional probing adsorbates are also used, but at smaller frequencies, enabling the quantification of surface area at varying temperatures and

measurement scales (Sing, 2001). These adsorbates include carbon dioxide, argon and water (Sing, 2001). Specific surface area has no single true value but it is a property that depends on the scale, so the values obtain are highly influenced by the adsorbate that is used and its adsorption cross section (Hanaor et al., 2014).

As the size of the particles decreases, the ratio of the surface area to the overall volume of the particle responds by increasing. This is useful in nanomaterials because as compared to bulk materials where most of the atoms are in the interior volume, for nanomaterials most atoms are on the surface (Brame & Griggs, 2016). Surface area that is accurately measured can also help to estimate transformation of a material as well as its toxicity (Hull et al., 2012; Kennedy et al., 2015). Properties that are unique to nano-materials can be more accurately determined by using specific surface area than just particle size (Kreyling et al., 2010). Hence, it is of great importance to accurately measure the specific surface area of nano-materials in order to determine their effect on the properties.

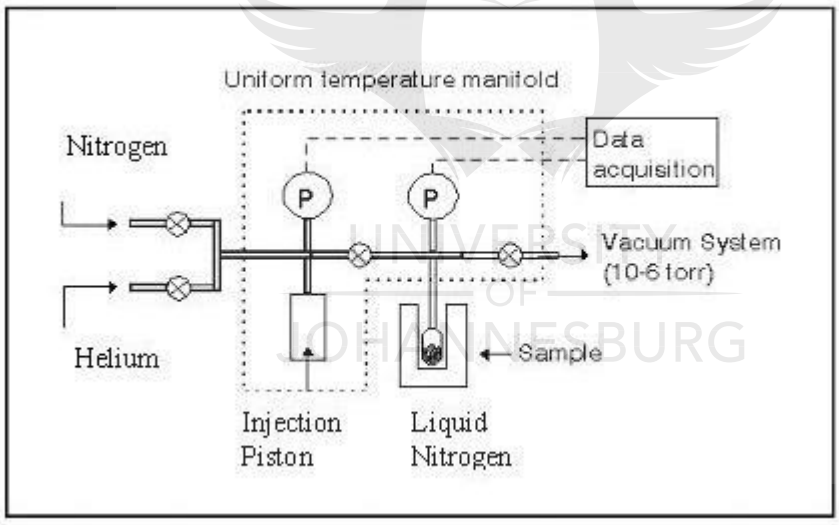

Fig. 2.5: Schematic representation of BET principle adopted from (Oxford, 2018)

The principle behind this instrument involves constant introduction of the standard amounts of the adsorbate material to the sample compartment, this process is kept at liquid nitrogen temperature which is -196.15 ℃ (Webb, 2003, Fig. 2.5). The constant injection of the gas on to the sample causes a slow decrease in pressure in the manifold until equilibrium is established. The injection system is composed of a calibrated piston, which is responsible for automatic

variation of the injection volume as well as the pressure but this process is dependent on the adsorption rate and the required resolution. Using this calibrated pistol method is an added advantage since it keeps the manifold dead volume constant as the system waits to reach pressure equilibrium. Even the smallest dead volume on the sample can make the instrument extremely sensitive to the amount of gas that is adsorbed on it (Webb, 2003).

BET is such a versatile technique with a wide variety of applications and it is used by multiple industries across the globe. This technique is used in catalysis, in this field the surface area of catalysts vital in determining the catalytic properties (Webb, 2003). It can also be used in nanoscience as the particle size as well as pore volume also determine the properties of nano materials (Srivastava, 2012). This technique can also be used in medicine, batteries, water purification, pharmaceutical industry, cosmetic industry and fuel cells (Acevedo et al., 2017; Hwang & Barron, 2015; Zielinski, 2013).

2.5.6 High performance liquid chromatography (HPLC)

High performance liquid chromatography (HPLC) is a powerful chromatographic technique that uses pressure to pump the mobile phase through the column. This technique can be used to separate and quantify components in a mixture to concentrations as low as parts per trillion (Paré and MichelSigouin 1997). Typical schematic diagram for an HPLC is shown in Fig. 2.6. In HPLC the mobile phase and stationary phase are immiscible; this makes it easy for the mobile phase to pass through it so it can interact with the stationary phase (Tswett & Chrome, 2018). The pump is used to push the mobile phase through the column (Mcpolin, 2009). This helps reduce the time it takes for the analyte to pass through. The sample is injected through the injector that is situated near the column inlet. The injected sample is pushed through the column by the pump passing through the stationery phase packed inside. The components that interact more with the stationary phase elute last while those with less interaction elute first (Mcpolin, 2009). This interaction is through dispersion, those that disperse in the mobile phase elute first and those that disperse in the stationary phase elute last. Elution is then detected by the detector that is connected to the outlet (Koerner, 2013). The outlet is composed of the data system and the waste container.

The data system collects and records all the data from the detector and displays them as numbers or a graph that is referred to as a chromatogram. These days, any information that can

prove to be useful, such as quality control, pharmacology, pharmacodynamics, pharmacokinetics, and toxicology of medicament can obtained from chromatographic analysis (Wada, 2011). HPLC can separate macromolecules, ionic species, labile natural compounds, polymeric materials (Tswett & Chrome, 2018). HPLC is a very flexible technique; its components can be changed to better suit the analysis. For example the column size, length and the detector (Malviya & Sharma, 2014).

The use of HPLC can be both qualitative and quantitative. It can be used for both compound quantification and identification. Reverse phase chromatography is the most common and only a few separations cannot be done using reverse phase. The development of this technique as well as its flexibility, speed, high efficiency and diversity is the main reason why it is used in various industries such as pharmaceutical, cosmetics, ceramics, medicine, forensics etc. Almost all science fields use this technique especially chemistry, these fields include food technology, polymer chemistry, nanotechnology, combinatorial chemistry, applied chemistry, environmental chemistry and green chemistry (Chang, 2016; Daldrup, Michalke, & Szathmary, 1986; Koel & Kaljurand, 2006; Mcpolin, 2009).

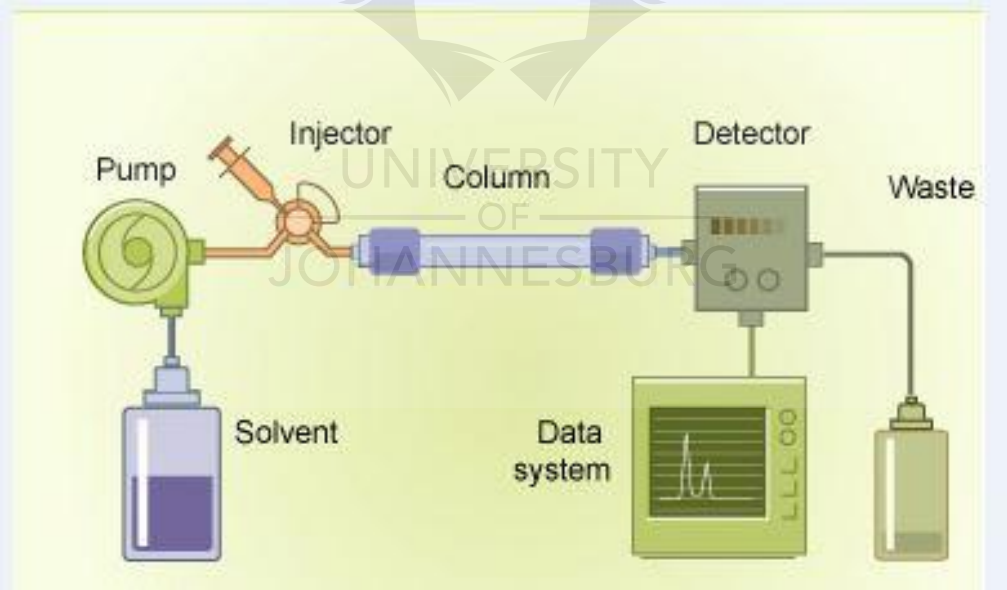

Fig. 2.6: Schematic representation of HPLC principle adopted from (Lingeman, 2018)

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CHAPTER 3:
ULTRASOUND ASSISTED DISPERSIVE MAGNETIC SOLID PHASE MICROEXTRACTION BASED ON SYNTHESISED Fe_3O_4-Al_2O_3@CNFs NANOCOMPOSITE COUPLED WITH HIGH PERFORMANCE LIQUID CHROMATOGRAPHY FOR EXTRACTION, PRECONCENTRATION AND QUANTIFICATION OF 17-BETA ESTRADIOL (E2) IN WASTEWATER

ABSTRACT

In this work, Fe_3O_4-Al_2O_3@CNFs nanocomposite was synthesised and applied in the ultrasound assisted dispersive magnetic solid phase microextraction (UA-DMSPME) of 17-beta estradiol (E2) in wastewater followed by their determination using high performance liquid chromatography coupled with diode array detector (HPLC-DAD). Several parameters (such as pH, mass of adsorbent, extraction time as well as eluent volume) affecting the efficiency of this sample preparation technique were optimised in order to achieve excellent sensitivity and high recoveries of E2. These parameters were optimised using response surface methodology based on Box Behnken design. Under optimised conditions, the linear dynamic range was achieved in the range of 0.83-1000 ng mL^{-1} with the correlation coefficient of 0.9951. The preconcentration factor, limit of detection (LOD) and limit of quantification (LOQ) were found to be 334, 0.25 ng mL^{-1} and 0.83 ng mL^{-1}, respectively. The intraday (repeatability, n = 10) and interday (reproducibility, n = 5 working days) expressed in terms of relative standard deviation (%RSD) were 0.8% and 3.3%, respectively. The developed UA-DMSPME/HPLC-DAD method was applied for the preconcentration and quantification of E2 in wastewater samples. The obtained results indicated that E2 was present in the wastewater samples.

Keywords: Carbon nanofibers, ultrasound assisted dispersive magnetic solid phase microextraction, 17 beta-estradiol, Response surface methodology, desirability function

3.1 INTRODUCTION

Water is essential for life, and no living organisms can survive without it and therefore, it is of utter most importance that every human being has access to clean and safe water (Muller, 2011). In most of the rural areas, people are deprived of the right to sufficient water due to lack of infrastructure and one major global problem that further decreases the amount of fresh water

is water pollution. The pharmaceutical industry is one of the fastest growing industries in the world which means that pharmaceutical pollution is also growing at a fast rate (Ebele, Abou-Elwafa Abdallah, & Harrad, 2017). This is problematic since most pharmaceutical pollutants fall under the emerging pollutants (EPs) category (Geissen et al., 2015). These are the type of natural or synthetic chemicals that are not commonly monitored or removed by conventional methods but can be found in the environment. Their presence can cause adverse ecological and human health effects (Geissen et al., 2015). Different types of pharmaceuticals such as hormones, antibiotics, contraceptives, antiepileptic to mention but few, have been identified as environmental pollutants (Shalini, Anwer, Sharma, Garg, & Kumar, 2010). Hormones and their supplements are also considered harmful to the environment because they can be endocrine disrupting chemicals. This group of chemicals are defined as those that may interfere with the hormone biosynthesis and metabolism systems at certain doses and cause several diseases and conditions such as cancer, reproductive problems, developmental disorders and immune effect (Diamanti-Kandarakis et al., 2009).

The hormone17-beta estradiol (E2) is one of the most commonly known steroid hormones that are produced naturally in humans and animals (Tanaka, Yakou, Takahashi, Higashitani, & Komori, 2001). This hormone is responsible for sexual reproduction in women but is also produced by men and it is found in the highest concentration in pregnant women (Sneader, 2006). It is also responsible for development of sexual characteristics in women (Havlíková et al., 2006). This hormone can also be taken as a supplement for women with hormone imbalance. It is commonly released into the environment through urination (Damstra, 2003) and women can produce about 2.4 µg per day while man can release about 1.5 µg in the same period (Damstra, 2003). But pregnant women on the hand release up to 259 µg in a single day (Johnson, Belfroid, & Di Corcia, 2000; Sun & Zhou, 2014). Urination is not the only path taken by this hormone to the environment as it can do so through faeces, vomit, pharmaceutical industries, pathology laboratories and hospitals. This hormone in turn can lead to adverse effect in humans and animals and aquatic life due to its metabolic properties (Zhang and Zhou 2005). Since E2 falls under the EPs category, most conventional waste water treatment methods cannot detect or remove it successfully (Sun & Zhou, 2014). This is raising a global concern for environmental pollution because such chemicals are only accumulating in the rivers and worst of, in wastewater treatment plants (Murdoch, 2015). For this reason, researchers have dedicated their time to find the best solution to resolve this problem.

Recently solid phase extraction (SPE) procedures are widely used for extraction and preconcentration of a wide range of analytes in environmental matrices (Andrade-Eiroa et al.,

2016; Ibrahim, Nodeh, & Sanagi, 2016). However, traditional SPE methods are lengthy, time-consuming and the columns are normally blocked by sample matrix (Satpathy,Tyagi, & Gupta, 2011). These challenges can be solved by application of dispersive solid phase extraction (Ebrahimpour et al., 2015; Hao et al., 2015; Zhao et al., 2016). The Advantages of DSPE include simplicity, rapidity, and inexpensive. This method has been applied for extraction and preconcentration different steroid hormones (Dil et al., 2017; Hao et al.,2015; Zhao et al., 2016). Similar to any SPE based method, in DSPME, the selection of an effective adsorbent that will lead to the attainment of satisfactory recovery and high enrichment factor is a very important factor. Several adsorbents have been used for extraction of hormones and these include ethylene diamine-functionalised magnetic carbon nanotubes (Zhao et al., 2016) iron-embedded porous carbon material (MIL-53-C) (Ma et al., 2016) core-shell magnetic covalent organic frameworks (Chen et al., 2018), magnetic imprinted nanoparticles (Hao et al, 2015): magnetic nano-polypyrrole (Ebrahimpour et al., 2015), cetyltrimethyl ammonium bromide (CTAB)-coated Fe_3O_4@caprylic acid NPs (Wang et al., 2016), magnetic carbon microparticles (González et al. 2017), core-shell polydopamine coated magnetic nanoparticles to name but few (Socas et al, 2018).

This work reports on the use of dispersive magnetic solid phase microextraction based on carbon nanofibers (CNFs) coated with iron oxide and aluminium oxide nanoparticles (Fe_3O_4-Al_2O_3@CNFs) as an adsorbent for preconcentration of E2 in wastewater. The quantification of the analytes was conducted using high performance liquid chromatography with a diode array detector (HPLC-DAD). Nano-Al_2O_3 was chosen due to its excellent nanofiltration properties, large surface area and high thermal stability (Tamura, Katayama, & Furuichi, 1997). The Fe_3O_4 nanoparticles were used because they have high chemical stability, super paramagnetic properties and non-toxicity (Yao et al. 2012; Chang and Chen 2005). Lastly, carbon nanofibers were chosen due to their large surface area, narrow pore size distribution, affinity towards organic pollutants and excellent adsorption capacity (Ahmed et al., 2015; des Ligneris, Dumée, & Kong, 2018). All these excellent properties were combined to produce a nanocomposite with high adsorption capacity, highly stable, non-toxic and easy to recover after adsorption. Parameters that can affect the extraction, preconcentration and recovery efficiency of E2 were optimised using multivariate approach.

3.2 EXPERIMENTAL

3.2.1 Materials and reagents

Iron(II) chloride tetrahydrate ($FeCl_2 \cdot 4H_2O$, 99%), 17-β Estradiol, methanol (HPLC, ≥99%), ethanol (HPLC, ≥99%), sodium hydroxide, ammonia and acetonitrile (HPLC, ≥99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solution of 17-beta estradiol (10 mg/mL) was prepared in ultra-pure water (Direct-Q® 3UV-R purifier system, Millipore, Merck). Working standards of were prepared daily by diluting appropriate volumes of the stock solution in ultra-pure water. A 100 mg L^{-1} standard stock solution of E2 was prepared by dissolving it with methanol in 50 ml volumetric flask and diluted to the mark with ultrapure water then the solution was stored at 4 °C. Working solutions (1 and 10 mg L^{-1}) were prepared by diluting with ultra-pure water appropriate volumes of stock solutions. Standard solution of 50-6000 ng mL^{-1} were prepared for the calibration of the instrument.

3.2.2 Instrumentation

An OHAUS starter 2100 pH meter (Pine Brook, NJ, USA) was used for pH adjustments of the reagents and to measure the pH of samples. Dispersion was carried out using ultrasound bath (Bandelin Sonorex Digitec, Bandelin electronic GmbH & Co. KG, Berlin, Germany). All the reaction were stirred using a Labcon 3075U hotplate and magnetic stirrer (Labdesign Engineering, Maraisburg, RSA) and all the drying was done using oven (EcoTherm, Labotec, Midrand, Johannesburg, South Africa).

3.2.3 Preparation of Fe_3O_4

Preparation of magnetite was adopted from the method reported by Nabiyouni et al., 2014, in which 3 g of $FeCl_2 \cdot 4H_2O$ was dissolved in 100 mL of distilled water. 45 mL of 5 mol L^{-1} NH_3 solution was added to the above solution drop wise under ultrasonic irradiation for 20 min. A black precipitate was obtained confirming the synthesis of Fe_3O_4. The precipitate of Fe_3O_4 was then centrifuged for 15 min and rinsed with distilled water followed by 3 mol L^{-1} NaOH to remove the excess NH_4Cl, subsequently the product was left in an oven at 60 °C for 12 hours to dry.

3.2.4 Preparation of Fe_3O_4-Al_2O_3@CNFs

Preparation of the nanocomposite was prepared according to previous studies with modification (Munonde, Maxakato and Nomngongo 2017). Briefly, 2.07 g of anhydrous aluminium chloride ($AlCl_3$) was dissolved in 100 mL of ethanol under vigorous stirring. Then, 2 g of magnetic Fe_3O_4 nanoparticles and 1 g of CNFs were then added into the solution followed by the drop wise addition of 60 mL of ammonia solution (25 % v/v). The formed gel was left to maturate for 24 h at room temperature before being washed with distilled water and 3 M NaOH to remove excess salts then dried at 60 °C in an oven for 12 h. The obtained powder was then grinded into a fine powder using a pulvirisette. To ensure the complete formation of Al_2O_3 the powder was calcined at 500 °C for 3 h.

3.2.5 Chromatographic system and conditions

An Agilent 1200 Infinity series HPLC equipped with a diode array detector (Agilent Technologies, Waldbronn, Germany) applied to perform the analyses. The mobile phase consisting of acetonitrile and water in the ratio of 45:55 (v/v) was pumped through the column (C18) at the flow rate of 1.0 ml min^{-1}. With an injection volume of 20 µL. The column oven compartment was maintained at 25 °C and the detection was carried out at a wavelength of 281 nm. The chromatograms for the calibration standards are shown in Fig. 3.1 and the average retention time for E2 was 5.4 minutes. Fresh working samples were prepared for each day and new calibration was constructed. The solutions were filtered through a phenomenex membrane of 0.45 mm pore size (25 mm filter) and transferred to an auto-sampler vial for analysis. Calibration curve was sketched using peak area versus elution time of working solutions in mobile phase.

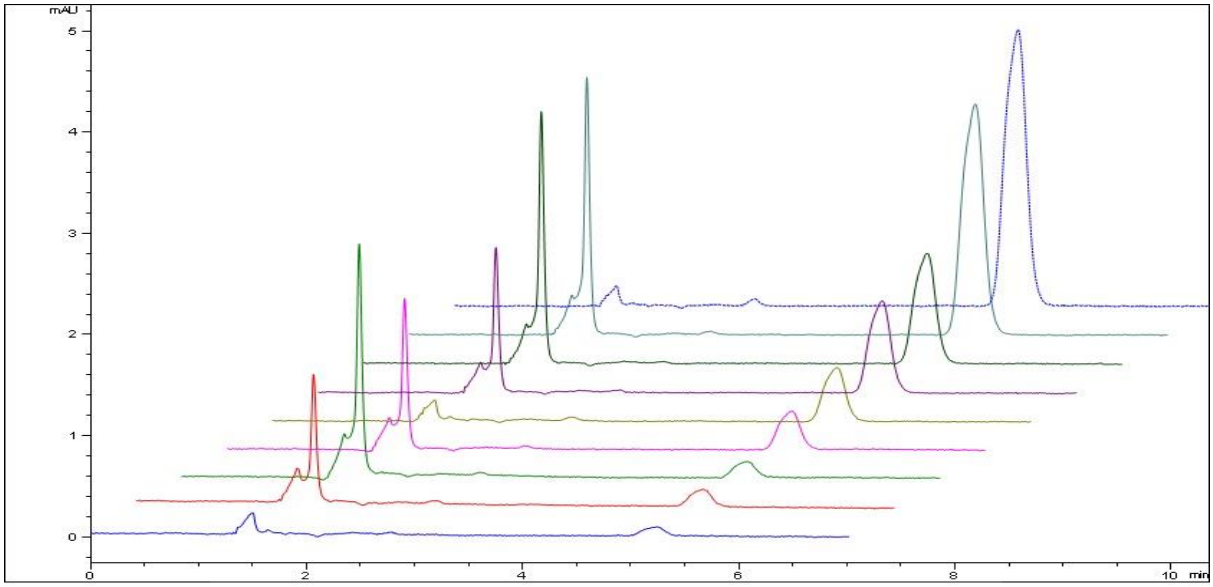

Fig. 3.1: Chromatograms of standards of E2 from 50-6000 ng mL^{-1}

3.2.6 Sampling and sample collection

The water samples used for analysis were collected Daspoort (Pretoria, Gauteng, South Africa) wastewater treatment plant. All the samples were collected in pre-cleaned 500 mL glass bottles. The samples were then refrigerated at 4 °C.

3.2.7 Ultrasound assisted dispersive magnetic solid phase microextraction procedure

The ultrasound assisted dispersive magnetic solid phase microextraction using the Fe_3O_4-Al_2O_3@CNFs nanocomposite as a sorbent was carried according to the previous studies reported by Han et al., 2012 with some modifications. Firstly, 20-60 mg of sorbent (Fe_3O_4-Al_2O_3@CNFs nanocomposites) was rinsed in 5 mL methanol and 5 mL deionised water. The adsorbent was dispersed into a 10 mL aqueous sample solution (pH 4-9, 100 µg L^{-1}) by ultra-sonication for 5-30 min to form a homogeneous suspension and to reach adsorption equilibrium. Afterwards a strong magnet was deposited at the bottom of the beaker to isolate the sorbent from the sample solution. After approximately 5 min, the suspension became limpid and the liquid was decanted. Then 500-100 µL of acetonitrile was added into the analytes then ultra-sonicated for a further 5 min. Finally, the strong external magnet was placed at the bottom of the beaker to separate the adsorbent and the eluent containing the analyte. The eluent was withdrawn into a syringe and filtered into a HPLC vial for analysis.

3.3 RESULTS AND DISCUSSION

3.3.1 Characterisation of the nanocomposite

3.3.1.1 Fourier Transform Infrared Spectroscopy (FTIR)

Fig. 3.2 shows the FTIR spectrum of (a) Al_2O_3, (b) Fe_3O_4, (c) Fe_3O_4- Al_2O_3@CNFs and (d) CNFs. The peaks at 512 and 469 cm^{-1} in Fig 3.2a were attributed to Al_2O_3 stretching while the peaks at 1636 cm^{-1} and 2930 cm^{-1} (Fig 3.2d) were attributed to the C-H bending of an alkene group in an aromatic ring and alkane group respectively of CNFs. The peaks at 1503, 3445, 3475, 3494 and 3578 cm^{-1} were assigned to the bending and stretching vibration mode of O-H bending and O-H stretching vibrations, which confirmed the presence of moisture adsorbed from the atmosphere. The band observed at 1486 cm^{-1} in Fig 3.2c was assigned to the bending vibration of free water molecule; while bands observed at 2360 and 1486 cm^{-1} (Fig 3.2c) may be attributed to -CH, and -CH_2 stretching vibrations possibly associated with CNFs in the Fe_3O_4- Al_2O_3@CNFs nanocomposite. Weak band observed at 1360 cm^{-1} may be due to C-H stretching vibrations from the CNFs. The peaks at 545 and 495 cm^{-1} in Fig 3.2c may be attributable to the Fe-O vibration bond. The above FTIR spectrum shows the successful incorporation of both Fe_3O_4 and Al_2O_3 on the surface of CNFs.

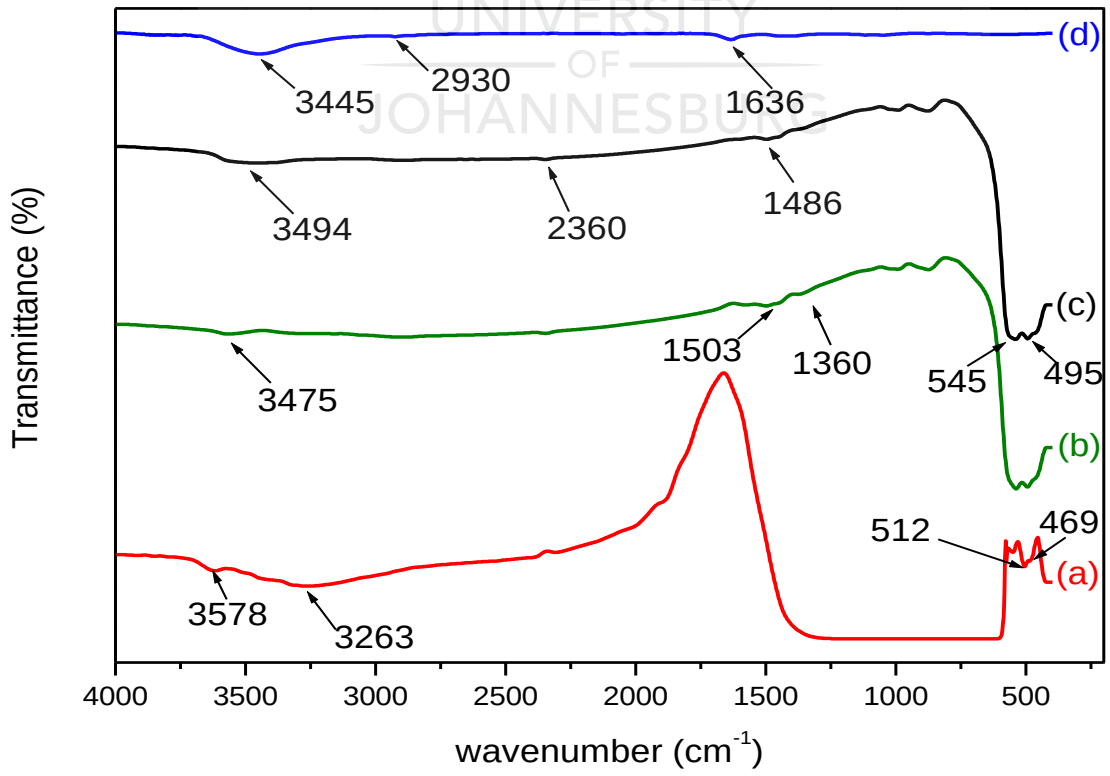

Fig. 3.2: FTIR spectrum of (a) Al_2O_3, (b) Fe_3O_4, (c) Fe_3O_4- Al_2O_3@CNFs and (d) CNFs.

3.3.1.2 X-ray Diffraction (XRD)

The x-ray diffraction pattern of (a) Al_2O_3, (b) CNFs, (c) Fe_3O_4- Al_2O_3@CNFs and (d) Fe_3O_4 are shown in Fig. 3.3 and their corresponding peaks are shown in **Appendix 3A-1**. Fig. 3.3a shows the oxide as aluminium oxide (Al_2O_3) and the XRD pattern is in full agreement with JCPDS 42-1468 (Sathyaseelan, Baskaran, & Sivakumar, 2013) and the crystals correspond to trigonal system with unit cell length as 4.81 Å. The crystallite sizes of the Al_2O_3, Fe_3O_4 and CNFs products were determined by using Debye Scherrer's formula:

$$D = \frac{0.9\lambda}{\beta cos\theta}$$

Where, K=0.9 is the shape factor, λ is the X-ray wavelength of CuKα1 radiation, β is the full width at half maximum (FWHM) of the peaks and θ is the glancing angle. The average crystallite sizes were found to be 28.8 nm (Al_2O_3), 27.5 nm (Fe_3O_4) and 12.3 nm (CNFs). Fig. 3.3d shows the oxide as magnetite (Fe_3O_4) and the XRD pattern is in total agreement with JCPDS 19-0629 (Iyengar et al., 2014) and the crystals correspond to cubic system with unit cell length as 8.32 Å. The Fe_3O_4 used was of face centered cubic system which is in full agreement with JCPDS 89- 4319 (Chen, Ni, & Chen, 2007). The broad peaks shown in Fig 3.3d indicate the ultra-fine nature and the tiny crystallite sizes of the Fe_3O_4 particles. Fig. 3.3c represents the Fe_3O_4-Al_2O_3@CNFs nanocomposites and all the peaks visible in Fig. 3.3a, b and d are present and this indicates the successful incorporation of Fe_3O_4 and Al_2O_3 on to the surface of CNFs.

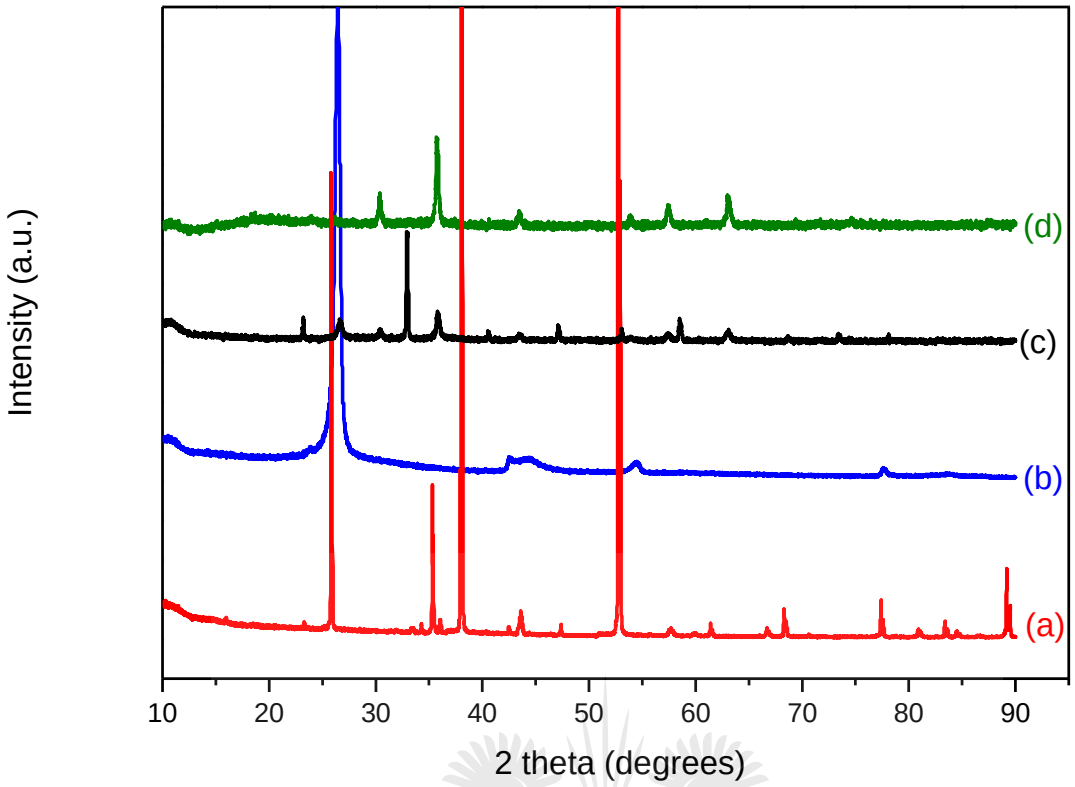

Fig. 3.3: XRD pattern of (a) Al_2O_3, (b) CNFs, (c) Fe_3O_4- Al_2O_3@CNFs and (d) Fe_3O_4.

3.3.1.3 Scanning electron microscopy/energy-dispersive spectroscopy (SEM/EDS)

The morphology of the carbon nanofibers is shown in Fig. 3.4a. The SEM images illustrate that the CNFs are typically cylindrical in shape but also consist of bamboo-shaped nanofibers. The SEM images also suggest that the length of the CNFs is up to several micrometres. The formation of the bamboo-shaped nanofibers shows that the carbon diffusion was not continuous with all fibers during their growth hence the inconsistency in fiber shapes, leading to a pulsed growth that translated into a periodic variation of the diameters of the nanofibers. The bamboo-shaped nanofibers are thicker as compared to the smooth fibers and as a result, they provide more surface area. Fig. 3.4b illustrates the morphology of Al_2O_3-Fe_3O_4 nanocomposite that is clearly visible in Fig. 3.4c which represent the Al_2O_3-Fe_3O_4@CNFs nanocomposite. This figure shows the Al_2O_3 and Fe_3O_4 scattered all over the surface CNFs. This is also supported by EDS in Fig. 3.4d which shows the elements present in the nanocomposite and their relative weight percent. The amount of carbon is displayed since the sample was carbon coated. Fe dominates with 59.5% followed by oxygen with 19.8% and Al with 13.5%. This further confirms the incorporation of Al_2O_3 and Fe_3O_4 on the surface of the CNFs.

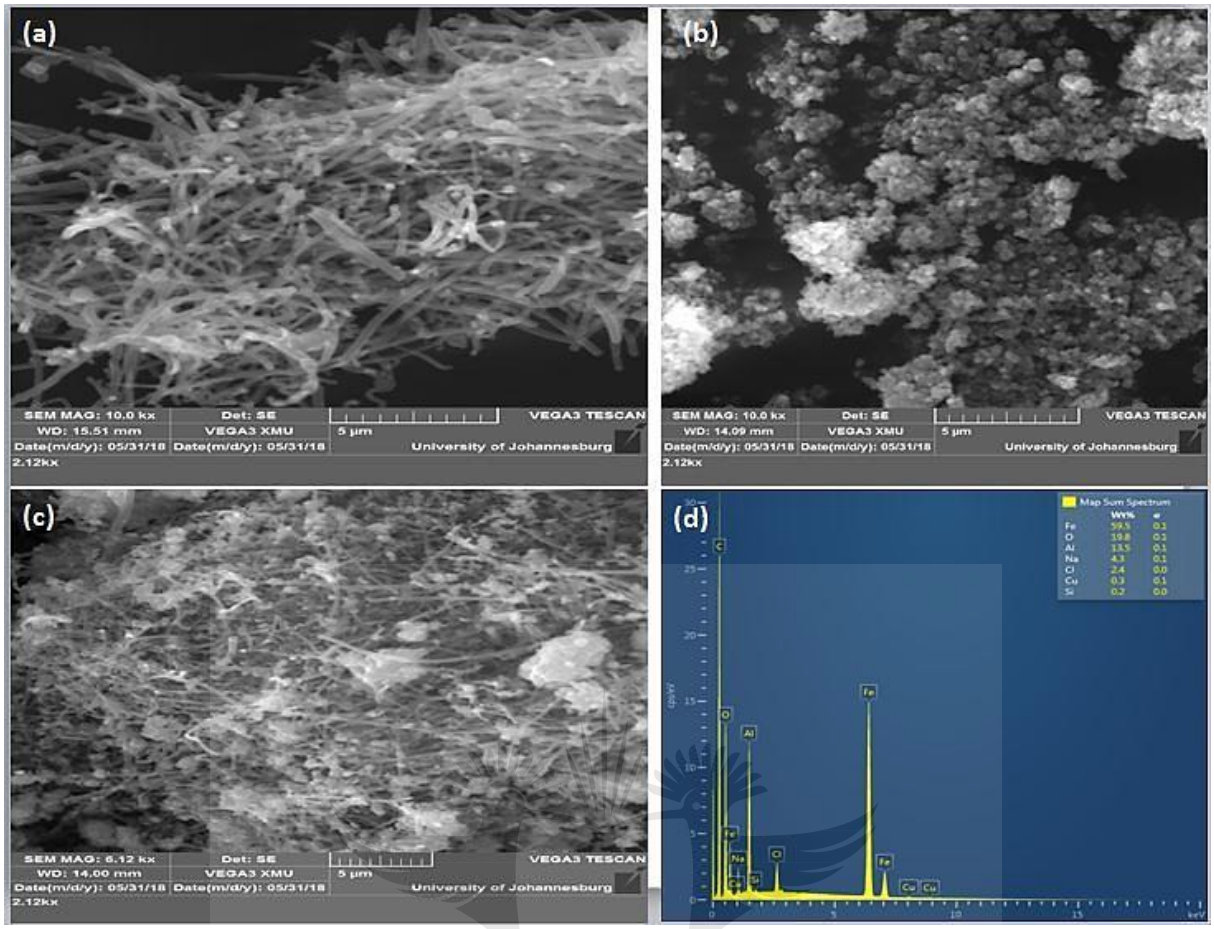

Fig. 3.4: SEM images of (a) CNFs, (b) Al_2O_3-Fe_3O_4, (c) Al_2O_3-Fe_3O_4@CNFs and (d) EDS of Al_2O_3-Fe_3O_4@CNFs.

3.3.1.4 Transmission Electron Microscopy (TEM)

The typical cylindrical and bamboo-shaped and smooth carbon nanofibers are illustrated in Fig. 3.5a. The TEM results are consistent with the SEM results and it can be observed that the cylindrical hollow fibers have smooth outer walls that are uniform and made of distinct sandwich of graphite layers. These graphite layers are parallel with respect to the axes of the fibers and display and almost defect free material. The bamboo-shaped fibers on the other hand display some areas of defects and change in diameter in different areas. This is because these types of fibers are composed of multi-walled graphite structures. Such bamboo-shaped fibers are composed of hollow segments of a size approximately 100 nm delaminated by curved stacking of carbon sheets. Fig 3.5b shows how the Al_2O_3 and Fe_3O_4 nanoparticles are dispersed onto the surface of CNFs. This figure shows how the Al_2O_3 and Fe_3O_4 nanoparticles increase the surface area of the CNFs. Fig. 3.5b also illustrate the different shapes of the nanoparticles. Fe_3O_4 crystallised in a cubic face-centred structure that is clearly visible on this figure. This is

in agreement with the XRD results obtained and supports the full incorporation of Al_2O_3 and Fe_3O_4 nanoparticles on to the surface of CNFs.

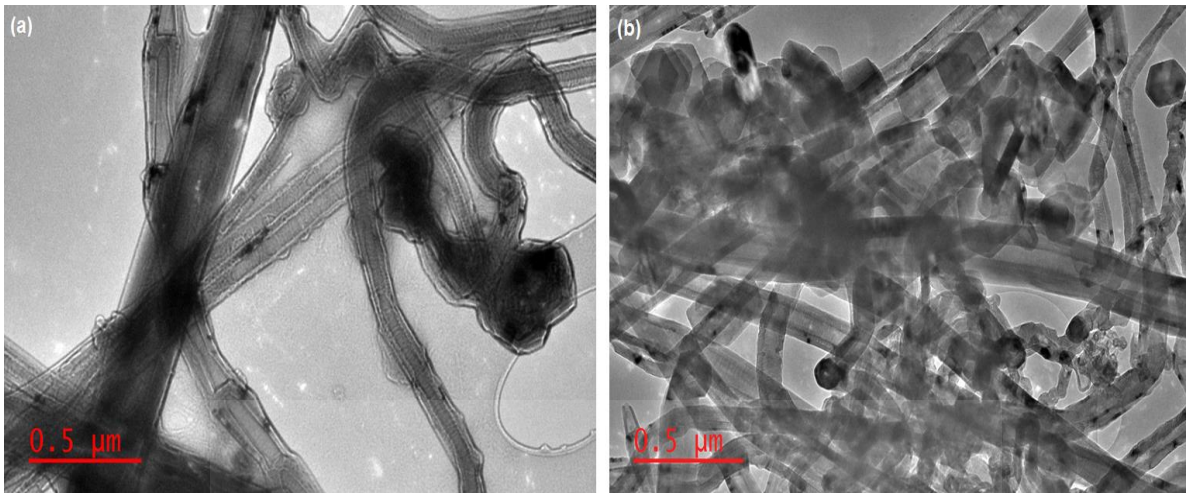

Fig. 3.5: TEM images of (a) CNFs and (b) Al_2O_3-Fe_3O_4@CNFs taken at 0.5 µm.

3.3.2 Optimisation of separation and preconcentration method

In order to achieve optimum conditions, the effect of eluent volume (EV), mass of adsorbent (MA), extraction time (ET), and pH were investigated using multivariate analysis. Firstly, fractional factorial screening (2^{4-1}) design with four central points was carried out to assess the most influential parameters that have significant effect on the analytical response (percentage recovery, %R). The factors that were found to have an influence on the analytical response were further optimised by response surface methodology based on Box-Behnken design.

3.3.2.1 Screening analysis using fractional factorial design

The fractional factorial design matrix and analytical response are presented in Table 3.1. The results in Table 3.1 were assessed using analysis of variance (ANOVA) and they were reproduced in the form of Pareto chart. As in Fig. 3.6, the Pareto chart indicated that the change in EV and ET did not have any significant impact on the percentage recovery. The sample pH and mass of the adsorbent were found to be significant at 95% confidence level. When comparing ET and EV, it can be seen that extraction time has some effect on the extraction and preconcentration of E2. Therefore, the effect of sample pH, extraction time and mass of adsorbent were further optimised by Box-Behnken design.

Table 3.1: Two level (2^{4-1}) fractional factorial design matrix and analytical response (%R)

Experiment No	EV/uL	MA/mg	ET/min	pH	%R
1	500	20	5	4	50.7
2	1000	20	5	9	17.9
3	500	60	5	9	13.7
4	1000	60	5	4	35.5
5	500	20	30	9	28.8
6	1000	20	30	4	67.6
7	500	60	30	4	33.9
8	1000	60	30	9	26.1
9 (C)	750	40	17.5	6.5	23.1
10 (C)	750	40	17.5	6.5	59.7
11 (C)	750	40	17.5	6.5	50.1
12 (C)	750	40	17.5	6.5	48.1

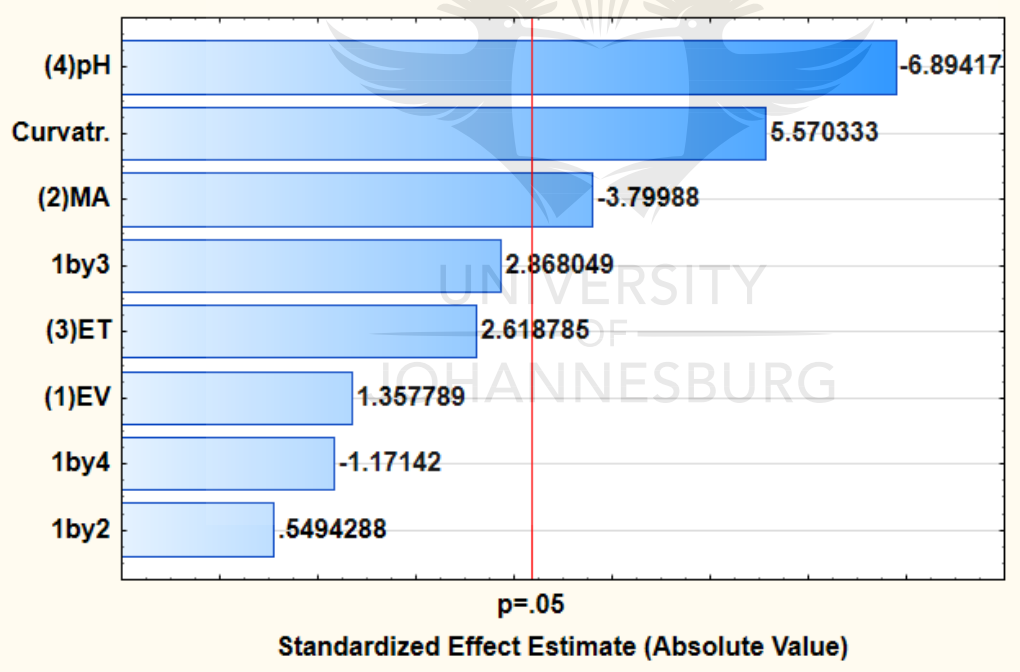

Fig. 3.6: Pareto chart for the full factorial design 2^{4-1} showing the standardised effect estimates of independent variables and their interaction.

3.3.2.2 Response surface methodology based on Box-Behnken design

Variables such as MA, pH and ET were optimised using a response surface methodology based on Box-Behnken to attain their critical values and the results are shown in Table 3.2. The 3-dimensional response surface plots showing the %recovery versus the process parameters plots

are represented in Fig. 3.7. The decrease in %R is due to the depletion of adsorption sites or lack of analyte binding sites on the surface of the adsorbent. Fig. 3.7a, b and c show that maximum %R is achieved at the extraction time of 24-30 min; this indicates that adsorption nature of the adsorbent was not that rapid. Fig. 3.7a and b indicate that sample pH values between 6 and 7, led to maximum extraction and preconcentration of E2. This phenomenon is supported by the %recovery increasing up to 100%. However, the % recoveries decreased at pH values lower or higher than 6-7. The reasoning behind this kind of behaviour can be attributed to protonation and deprotonation of surface functional groups of E2 as well as the adsorbent material. E2 has a net charge of zero; hence, it binds best in neutral conditions. Hence the decrease in %R in both acidic and alkaline conditions.

Table 3.2: Box-Behnken design matrix and analytical response for optimization of preconcentration of E2 by UA-DMSPME/HPLC-DAD method.

Experiment No	**pH**	**MA**	**ET**	**%R**
1	4	20	17.5	78.4
2	9	20	17.5	68.0
3	4	60	17.5	74.7
4	9	60	17.5	72.4
5	4	40	5	62.9
6	9	40	5	57.5
7	4	40	30	82.5
8	9	40	30	67.5
9	6.5	20	5	88.7
10	6.5	60	5	88.4
11	6.5	20	30	95.1
12	6.5	60	30	100.7
13	6.5	40	17.5	96.1
14	6.5	40	17.5	96.6
15	6.5	40	17.5	96.6

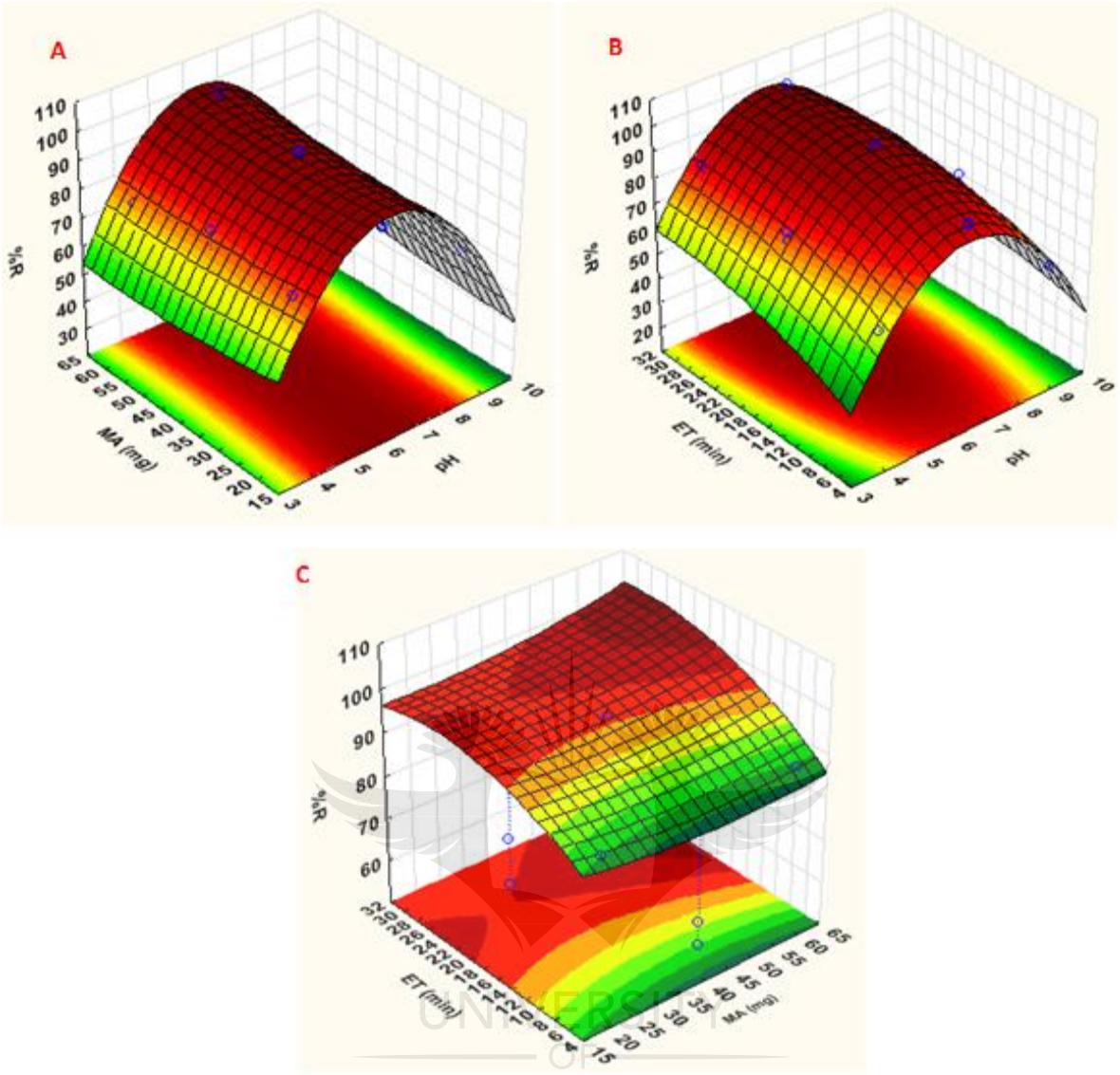

Fig. 3.7: Response surface and contour plots of the combined effects of (a) mass of adsorbent and pH, (b) extraction time and pH and (c) extraction time and mass of adsorbent.

In order to obtain the optimum condition, desirability profile (Fig. 3.8) was examined. As can be seen form Fig. 3.8, maximum recoveries could be obtained when sample pH, mass of Fe_3O_4-Al_2O_3@CNFs and extraction time were 6.5, 60 mg, 24 min. The performance of the adsorbent was found best at these conditions and they were used for real sample treatment. The optimum conditions were confirmed experimentally and the obtained experimental value (N = 5) and predicted value of E2 recovery were 99.9± 1.1% and 101%, respectively. Based on t-test these results were in good agreement with each other, suggesting that the model was valid.

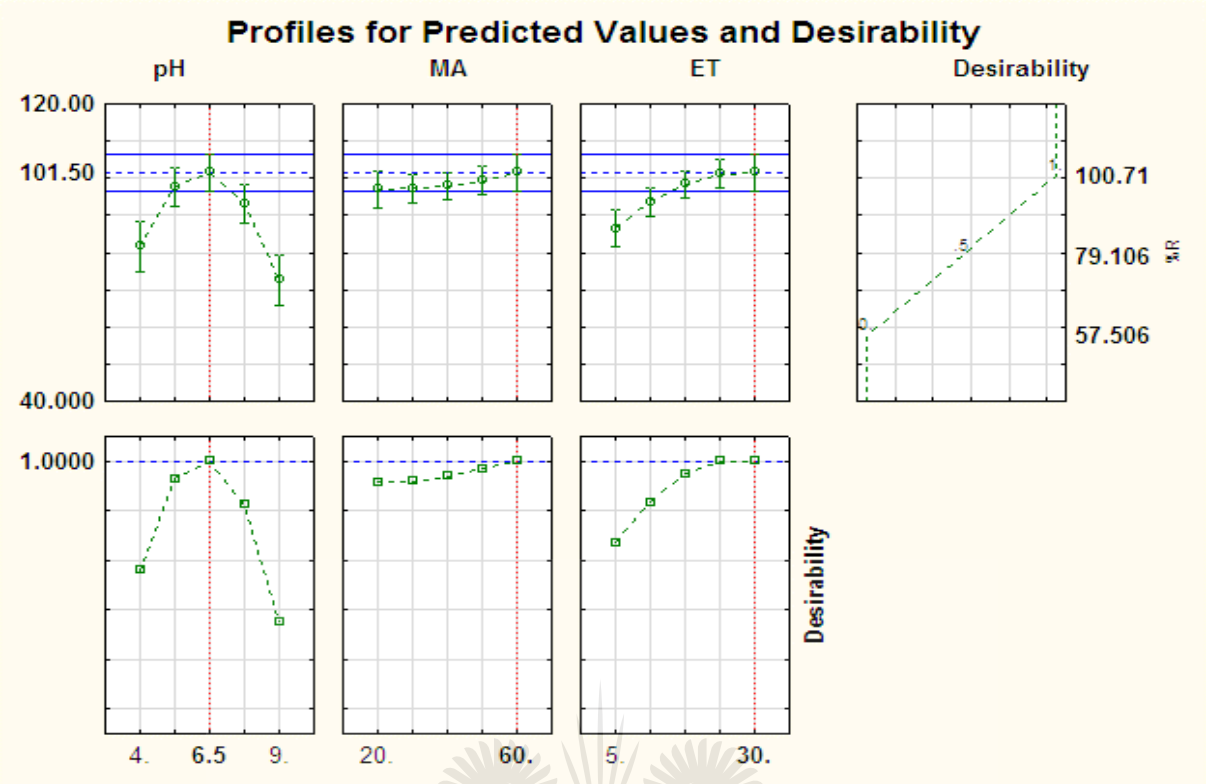

Fig. 3.8: Profiles for experimental and predicated values and desirability function for the recovery of E2.

3.3.3 Analytical performance of the UA-DMSPME-HPLC method

The performance of UA-DMSPME/HPLC-DAD was investigated at optimum conditions using parameters such as sensitivity (limits of detection (LOD) and quantification (LOQ)), linearity (linear range), precision (repeatability and reproducibility) and extraction efficiency (% recovery and preconcentration factor). The results are presented in Table 3.3 and they suggest reasonable linearity that was found to be from the LOQ to 1000 ng mL^{-1}. Precisions (repeatability and reproducibility) were evaluated analysing effluent wastewater sample spiked with 100 µg L^{-1} of E2. Both the repeatability (intraday, n = 10 consecutive determinations within the same day) and reproducibility (interday, n = 5 working days) were expressed as the relative standard deviation (%RSD). As seen in Table 3.3, the RSDs of less than 5.89 % were obtained indicating that the developed method had reasonable precision for preconcentration of E2 in complex matrix. The LOD and LOQ values for E2 are shown in Table 3.3 and they were calculated using the lowest concentration level on the calibration curve that yielded a signal-to-noise ratio of 3 and 10. The preconcentration factor defined as the slope of the calibration after preconcentration divided by the slope of the curve before preconcentration, was found to be 334.4.

Table 3.3: Analytical figures of merit of the DMSPME/HPLC-DAD method for preconcentration and determination of E2.

Analytical performance parameters	Values
Regression equation	$y = 2.4745x + 2.4058 : R^2 = 0.9951$
Linear range (ng mL^{-1})	0.83-1000
Limit of detection (LOD) (ng mL^{-1})	0.25
Limit of quantification (LOQ) (ng mL^{-1})	0.83
Repeatability (RSD, %) (N = 10)	0.8
Reproducibility (RSD, %) (N = 5)	3.3
Preconcentration factor (PF)	334.4

3.3.4 Validation and application

The accuracy of the DMSPME/HPLC-DAD procedure was evaluated using a wastewater samples spiked and unspiked from Daspoort (Pretoria, Gauteng, South Africa) wastewater treatment plant. The water samples were spiked using two different concentrations as shown in Fig. 3.9 (effluent wastewater sample). The analytical results are shown in Table 3.4 and the recoveries for the spiked samples were between 99% and 101%. These results confirmed the applicability and validity of the DMSPME/HPLC-DAD method for analysis of complex matrices. In addition to the spiked samples, two more samples were analysed for the presence of E2. The results obtained revealed that effluent sample 2 the concentration of E2 was about 55±1 ng mL^{-1}. While in the influent sample 2 the concentration of E2 below 561±7 ng mL^{-1}.

Table 3.4: Recoveries of E2 from wastewater samples spiked at two levels (100 ng mL^{-1} and 500 ng mL^{-1}) using the with the proposed DMSPME/HPLC method, n = 3

Sample	Added (ng mL^{-1})	Found (ng mL^{-1})	Recovery
Effluent 1	0	Below LOQ	-
	100	99.6±2	99.6
	500	505±6	101
Influent 1	0	642±8	-
	100	741±10	99.0
	500	1141±20	99.8

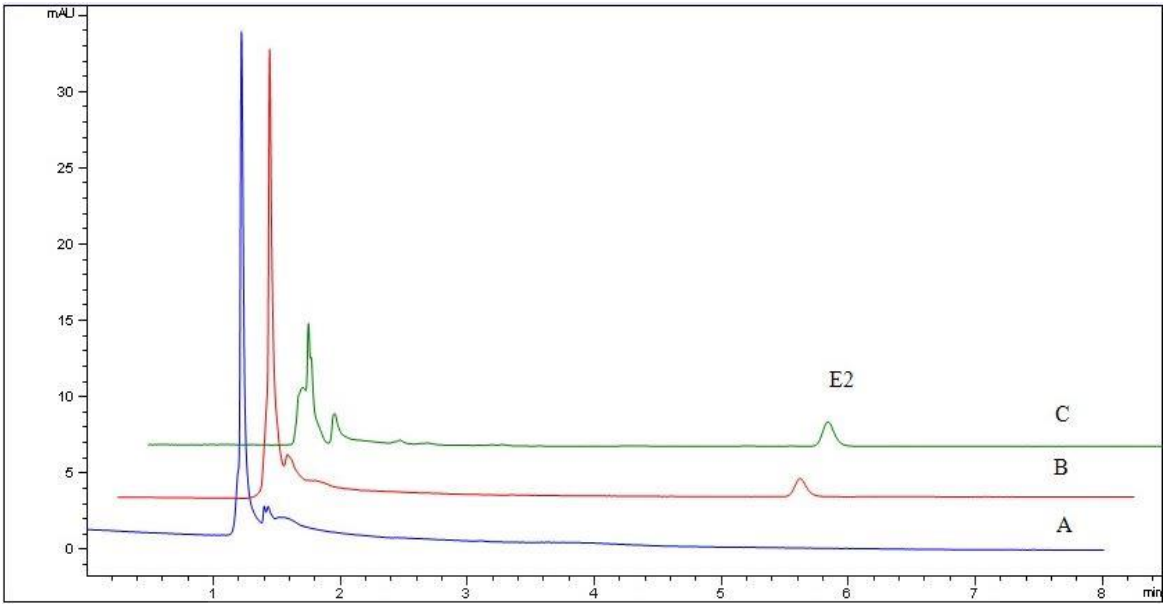

Fig. 3.9: Chromatograms of effluent wastewater sample (A) unspiked, (B) spiked with 100 ng/mL and (C) spiked with 500 ng/mL of E2 from Daspoort (Pretoria, Gauteng, South Africa) wastewater treatment plant.

3.4 CONCLUSIONS

In this paper, a UA-DMSPME extraction method coupled with HPLC-DAD was developed, tested and validated as a new approach for the efficient extraction and determination of E2 in wastewater samples. Operational parameters were optimised using factorial design approach. The developed extraction method DMSPME/HPLC-DAD was successful in extracting E2 in water samples with high efficiency and resulted in low detection limit, a wide linear range, and excellent recoveries with high accuracy and precision. The application of the proposed method to real samples was satisfactory and E2 have been detected in wastewater samples with concentrations ranging from 0 to 642 ng mL^{-1}.

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CHAPTER 4:
SYNTHESIS AND USE OF MgO-ZnO/CARBON NANOFIBER NANOCOMPOSITE AS AN ADSORBENT FOR ULTRASOUND ASSISTED DISPERSIVE SOLID-PHASE MICROEXTRACTION OF CARBAMAZEPINE (CBZ) FROM WASTEWATER PRIOR TO HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC DETECTION

ABSTRACT

A simple, rapid and efficient ultrasound assisted dispersive solid phase microextraction (UA-DSPME) method was developed for the preconcentration of carbamazepine (CBZ) in wastewater prior to HPLC-DAD determination. The MgO-ZnO@CNFs nanocomposite was used as an efficient adsorbent in magnetic dispersive solid-phase microextraction method. The structural and morphological properties of the nanocomposite were characterised by scanning electron microscopy and energy dispersive spectroscopy, transmission electron microscopy, X-ray diffractometer and Fourier transform infrared spectroscopy. The surface area was investigated using Brunauer–Emmett–Teller. Several factors (such as pH, mass of adsorbent, extraction time as well as eluent volume) that affect extraction and preconcentration of CBZ were also assessed and optimised using response surface methodology based on central composite design. Under optimal conditions, the limits of detection (LOD) and quantification were 0.08 µg L^{-1} and 0.29 µg L^{-1}, respectively. The calibration curve constructed after preconcentration of seven successive standards was linear in the concentration range of 0.3–800 µg L^{-1} with the correlation coefficient of 0.9922. The intra-day and inter-day precisions expressed in terms of relative standard deviation were 1.4% and 4.2%. A preconcentration factor of 490 was achieved and the method was applied for analysis of spiked wastewater. Satisfactory recoveries ranging from 97.8% to 102% were obtained.

Keywords: MgO-ZnO@CNFs nanocomposite, Dispersive solid phase microextraction; Carbamazepine; Desirability function.

4.1 INTRODUCTION

In the last decades, there has been more reports of pharmaceutical compounds that are present in rivers and surface waters at large (Andreozzi, Marotta, Pinto, & Pollio, 2002). The ever increasing demand of medicine for treatment of various diseases and disorders in veterinaries and humans has been recognised as the major source of these compounds that are polluting our

waters (Halling-Sørensen et al., 1998). When these pharmaceuticals are consumed, they are metabolised by the body and excreted out through defecation and urination as glucuronides and sulphates. However, some of them remain intact and are not converted to other compounds (Ternes, 1998). Besides the substances that remain metabolised, numerous other substances are transformed into active metabolites (Hoff et al., 2015). Defecation and urination are not the only sources of this type of environmental pollution but inappropriate disposal of unused and expired medicine also contribute to pollution (Murdoch, 2015). Some of these pharmaceutical compounds or their metabolites are very stable and mobile in natural environmental conditions (Murdoch, 2015). Since the water treatment plants fail to remove them, they can deposited into the soil which may reach the agricultural land (Larsen et al., 2004). Compounds that reach the surface water are likely to contaminate sources that are used for drinking water (Miao, Yang, & Metcalfe, 2005). Through intensive studies all over the world in the last few decades, some pharmaceutical compounds have been successfully detected in groundwater (Heberer, 2002b, 2002a; Sacher et al., 2001).

One of the widely used drugs for treatment of disorders is carbamazepine (CBZ). Generally CBZ is defined as a synthetic drug that falls under the benzodiazepine class and it is primarily used for treating epileptic disorders (Raghavendra et al. 2017). This drug does not only help epileptic patients control seizures but it is also used for treatment of bipolar, nerve disorders psychiatric therapy and it can also be used in trigeminal neuralgia treatment (Chen & Lin, 2012; Okuma, 2002). This drug is primarily digested by bile which is a fluid released from the liver where it is partially metabolised into CBZ 10, 11 epoxides and other derivatives (Swart et al., 1998). Similar other pharmaceutical drugs, its main sources are defecation, urination and improper disposal of expired or unused. When an average person takes CBZ, about 2 to 3% of it remains as a parent compound and it is released through urination and flushed down where it is mixed with wastewater (Clara, Strenn, & Kreuzinger, 2004; Sattelberger, 1999). Studies that were conducted in Europe and North America have indicated that this drug has been detected most frequently in wastewater treatment plants effluents and river streams (Heberer, 2002a; Metcalfe et al. 2003; Ollers et al., 2001; Ternes, 1998). CBZ have been found in high concentrations, up to several ng L^{-1} in surface waters (Clara et al., 2004; Ternes, 1998). In Germany, this drug has been detected at a concentration of 1075 ng L^{-1} in the surface waters of the city of Berlin (Heberer, 2002a).

The development of quick, accurate, affordable, easy to use and sensitive methods for detection, preconcentration and removal of CBZ at trace levels is necessary and has been one of the greatest challenges faced by analytical chemist for years. Recent analytical methods are

based mostly the use of on liquid chromatography-tandem mass spectrometry (LC–MS/MS) (Lübbert et al., 2017). This is because this technique is highly sensitive, can detect and identify compounds at extremely low concentrations but it is very complicated and expensive so not all the labs can afford it (Gros, Petrović, & Barceló, 2006). Therefore, several sample preparation methods have been developed to increase the detection limits of analytical detection techniques such as HPLC coupled with a UV detector (Vosough & Ghafghazi, 2014) and gas chromatography-flame ionization detector (Bahmaei, Khalilian, & Mashayekhi, 2018). These methods include solid phase microextraction (SPME) (Asgari et al., 2017), solid phase extraction (SPE) (dos Santos, Kakazu, Santos, Silva, & Figueiredo, 2017; Fortuna et al., 2010; Mirjana et al., 2012; Patrolecco et al., 2013): ultrasound-assisted emulsification microextraction (UAEME) dispersive liquid–liquid microextraction (DLLME) (Behbahani et al., 2013) and solid-phase extraction combined with dispersive liquid–liquid microextraction (Rezaee & Mashayekhi, 2012). Among the above-mentioned sample preparation techniques, dispersive solid-phase microextraction (DSPME) has attached significant attention due to attractive properties such as rapidity, less solvent consumption, simplicity and economical (Asfaram et al., 2015). Similar to traditional SPE, the nature of adsorbent play fundamental role in the extraction and preconcentration process (Asfaram et al., 2015).

Therefore, in this work a much cheaper, fast and simpler method utilising a preconcentration procedure based on ultrasound dispersive solid phase microextraction (DSPME) for analysis CBZ in wastewater. The analyte was quantified using high-performance liquid chromatography coupled with diode array as a detector (HPLC–DAD). The success of the UA-SPME method was by use ZnO-MgO@CNFs nanocomposite as an adsorbent. The influential experimental variables such as the sample pH, eluent volume, mass of adsorbent and extraction time were investigated and optimised using response surface methodology based on central composite design and desirability function.

4.2 EXPERIMENTAL

4.2.1 Material and reagents

All chemicals were of analytical reagent grade unless otherwise stated and ultra-pure water (Direct-Q® 3UV-R purifier system, Millipore, Merck) was used throughout the study. Carbamazepine, carbon nanofibers, sodium hydroxide methanol, $Mg(NO_3)_2 \cdot 6H_2O$, ethanol, $Zn(AC)_2$ and acetonitrile (HPLC, ≥99%) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Stock solutions of carbamazepine (10 mg/mL) were prepared in ultra-pure water

(Direct-Q® 3UV-R purifier system, Millipore, Merck). Working standards of 1000 ng/mL were prepared daily by diluting appropriate volumes of the stock solution in ultra-pure water. A 100 mg L^{-1} stock solution of CBZ was prepared with by dissolving appropriate amounts in small amounts of methanol, diluted with ultrapure water and stored at 4°C. Working solutions (1 mg L^{-1}) was used to prepared calibration standards ranging from of 50-6000 ng mL^{-1}.

4.2.2 Instrumentation

An OHAUS starter 2100 pH meter (Pine Brook, NJ, USA) was used for pH adjustments of the reagents and to measure the pH of samples. Dispersion was carried out using ultrasound bath (Bandelin Sonorex Digitec, Bandelin electronic GmbH & Co. KG, Berlin, Germany). All the reaction were stirred using a Labcon 3075U hotplate and magnetic stirrer (Labdesign Engineering, Maraisburg, RSA) and all the drying was done using oven (EcoTherm, Labotec, Midrand, Johannesburg, South Africa).

4.2.3 Synthesis of ZnO-MgO@CNFs nanocomposite

The synthesis of the nanocomposite was carried out according to previous studies with some modification (Aslani et al., 2018). To describe the method briefly, a 10 mmol solution of $Zn(AC)_2$ was dissolved in 50 mL of methanol and added to a 10 mmol solution of $Mg(NO_3)_2 \cdot 6H_2O$ that was also dissolved in 50 mL of methanol. Then 4 g of CNFs was added to the mixture. The mixture was then sonicated for 15 min to disperse the CNFs in the solution. 10 mL of sodium hydroxide solution (10 mol L^{-1}) was then added to the above mixture at room temperature under magnetic stirring. After 30 min of stirring the mixture was transferred into Teflon-lined stainless steel autoclaves, sealed, and maintained at 160 °C for 12 h. Subsequently, the reactor was cooled down to room temperature. The resulting solid products were centrifuged and subsequently washed with distilled water and methanol to remove the ions possibly remaining in the final products. The resultant product was dried at 60 °C in an oven for 12 hours followed by calcination at 500 °C for 2 hours to ensure proper formation of ZnO and MgO.

4.2.4 Chromatographic conditions

An Agilent 1200 Infinity series HPLC equipped with a diode array detector (Agilent Technologies, Waldbronn, Germany) was used perform the analyses. The mobile phase consisting of acetonitrile and ultra-pure water in the ratio of 30:70 (v/v) was pumped through

the column (C18) at the flow rate of 1.0 mL min^{-1}.With an injection volume of 10 µL. The column oven compartment was maintained at 25°C and the detection was carried out at a wavelength of 220 nm. Fresh working samples were prepared for each day and new calibration was constructed. The solutions were filtered through a phenomenex membrane of 0.45 mm pore size (25 mm filter) and transferred to an auto-sampler vial for further analysis. Calibration curves were constructed using peak area versus concentration of the standard solutions. The chromatograms for the standard solution are shown in Fig. 4.1 and the retention time of 9.2 minutes.

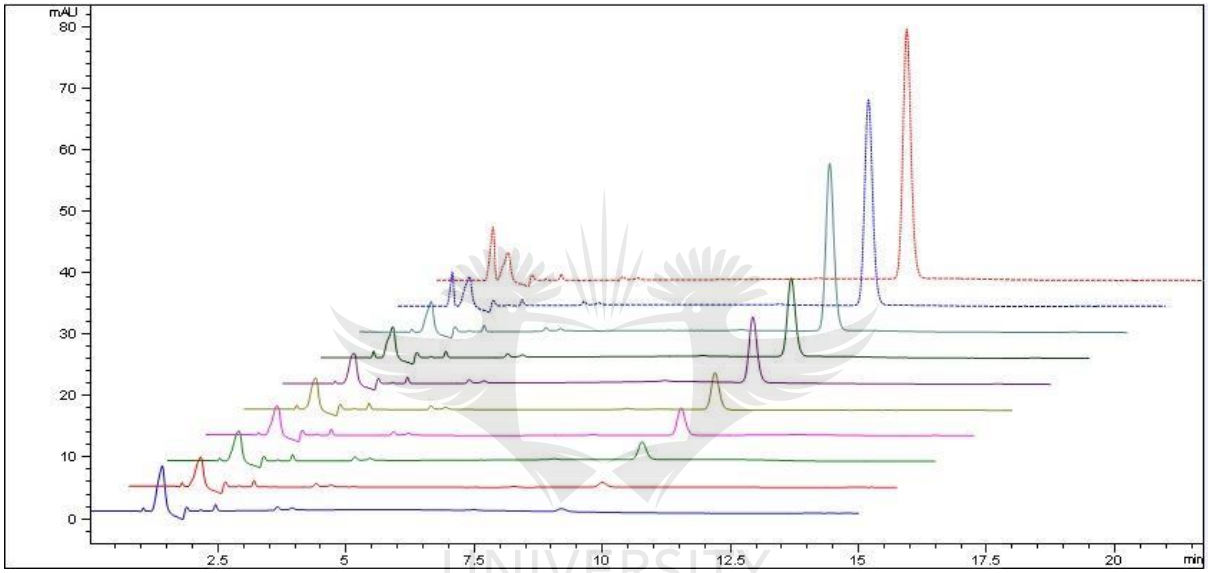

Fig. 4.1: Typical chromatograms for the CBZ calibration standards ranging from 50 to 6000 ng mL^{-1}

4.2.5 Sampling and sample collection

Influent (after sediment removal) and effluent wastewater samples were collected from Daspoort (Pretoria, Gauteng, South Africa) wastewater treatment plant. All the samples were collected in pre-cleaned 500 mL glass bottles. The samples were then refrigerated at 4°C

4.2.6 Ultrasound assisted dispersive solid phase microextraction procedure

The extraction and preconcentration procedure based on UA-DSPME was carried according to the previous studies (Nyaba et al., 2016). About 20-60 mg of ZnO-MgO@CNFs nanocomposite was first rinsed with 5 mL methanol followed 5 mL of ultrapure water. The adsorbent was then dispersed by ultra-sonication into 10 mL aqueous sample solution

containing 100 ng L^{-1} of CBZ (sample pH 4-9) for 5-30 min to form a homogeneous suspension. The adsorbent and aqueous phase were separated centrifugation at 3600 rpm for 5 min and the liquid was decanted. Then 500-1000 µL of acetonitrile was added into the solution containing the analytes then ultra-sonicated for a further 5 min. Finally, the eluent and the adsorbent were separated by centrifugation for 5 min and the separated solution containing the analyte was filtered into a HPLC vial followed by HPLC for analysis**.**

4.3 RESULTS AND DISCUSSION

4.3.1 Characterization

4.3.1.2 X-ray Diffraction (XRD)

Fig. 4.2 shows the XRD patterns of (a) CNFs, (b) ZnO, (c) MgO-ZnO@CNFs nanocomposite and (d) MgO (their characteristic peaks shown in **Appendix 4A-1**. The XRD pattern Fig 4.2a shows the CNFs spectrum with characteristic peak at 25.9º and this peak can also be observed on Fig 4.2c which is the nanocomposite. Fig 4.2b represents the XRD pattern of ZnO which is in full agreement with JCPDS 36-1451 (Dinesh et al., 2014) and the crystals correspond to hexagonal system with unit cell length as 3.2539 Å. The crystallite sizes of the ZnO, MgO and CNFs products were determined by using Debye Scherrer's formula:

$$D = \frac{0.9k\,\lambda}{\beta\,\mathrm{Cos}\theta}$$

Where, K=0.9 is the shape factor, λ is the X-ray wavelength of CuKα1 radiation, β is the full width at half maximum (FWHM) of the peaks and θ is the glancing angle.

The average crystallite sizes were found to be 36.3 nm (ZnO), 26.9 nm (MgO) and 12.3 nm (CNFs). Fig 4.2d shows the oxide as magnesium oxide and magnesium hydroxide (MgO). This XRD is in total agreement with JCPDS 89-7746 (Dhineshbabu et al., 2014) and the crystals correspond to cubic system with unit cell length as 4.21 Å. The broad peaks resonating at (111), (200), (220), (311) and (222) diffraction planes show the ultra-fine nature and the tiny crystallite sizes and reveal the cubic phase of MgO nanoparticles. Fig 4.2c represents the MgO-ZnO@CNFs nanocomposites and all the peaks visible in Fig 4.2a, b and d are seen on this figure. This indicates the successful incorporation of MgO and ZnO on to the surface of CNFs.

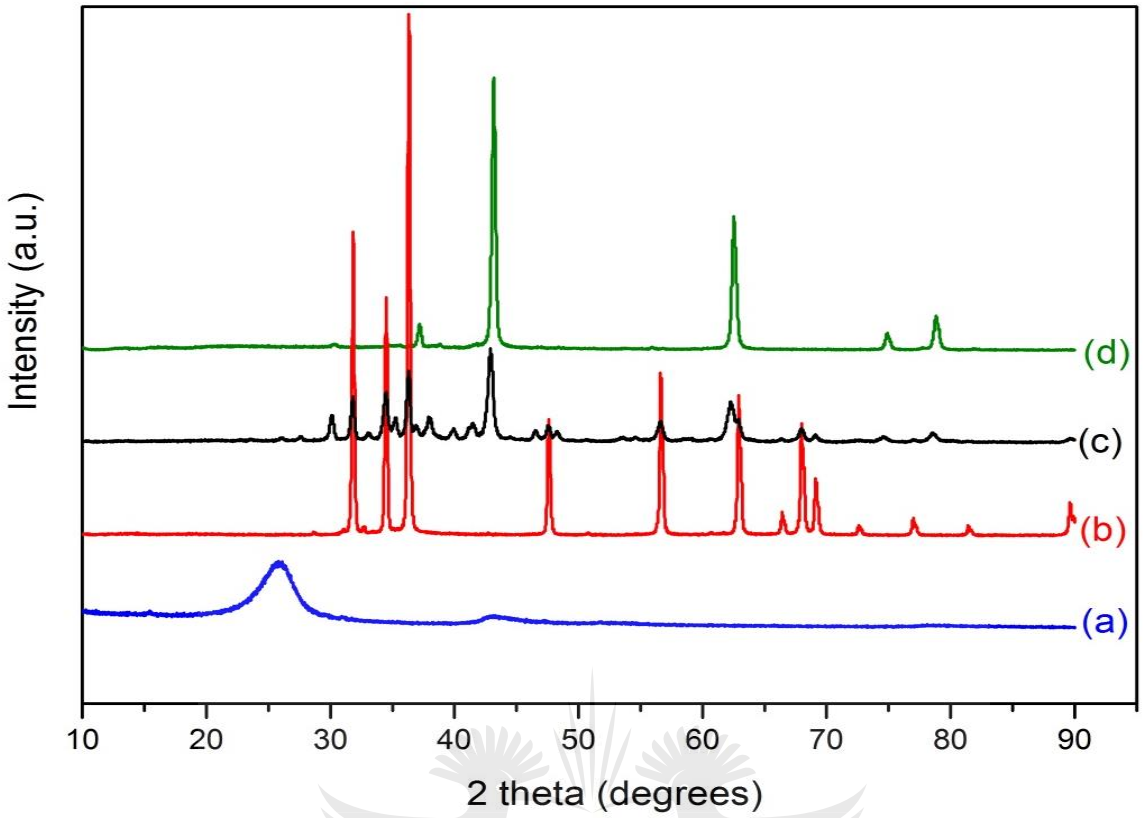

Fig. 4.2: XRD pattern of (a) CNFs, (b) ZnO, (c) MgO-ZnO@CNFs and (d) MgO.

4.3.1.2 Scanning electron microscopy (SEM) and energy-dispersive spectroscopy (EDS)

The morphology of the carbon nanofibers is shown in Fig. 4.3a and the SEM image illustrated that the CNFs are typically cylindrical in shape but also consist of bamboo-shaped nanofibers. The SEM images also suggest that the length of the CNFs is up to several micrometres. Fig. 4.3b illustrates the porous structures of MgO-ZnO nanocomposite that is also visible in Fig. 4.3c which represent the MgO-ZnO@CNFs nanocomposite. It can be seen from Fig. 4.3c s that the ZnO-MgO nanoparticles were scattered onto the surface CNFs. This is also supported by EDS in Fig. 4.3d which shows the elemental composition present and their relative weight percent. These results further confirm the incorporation of ZnO-MgO on the surface of the CNFs.

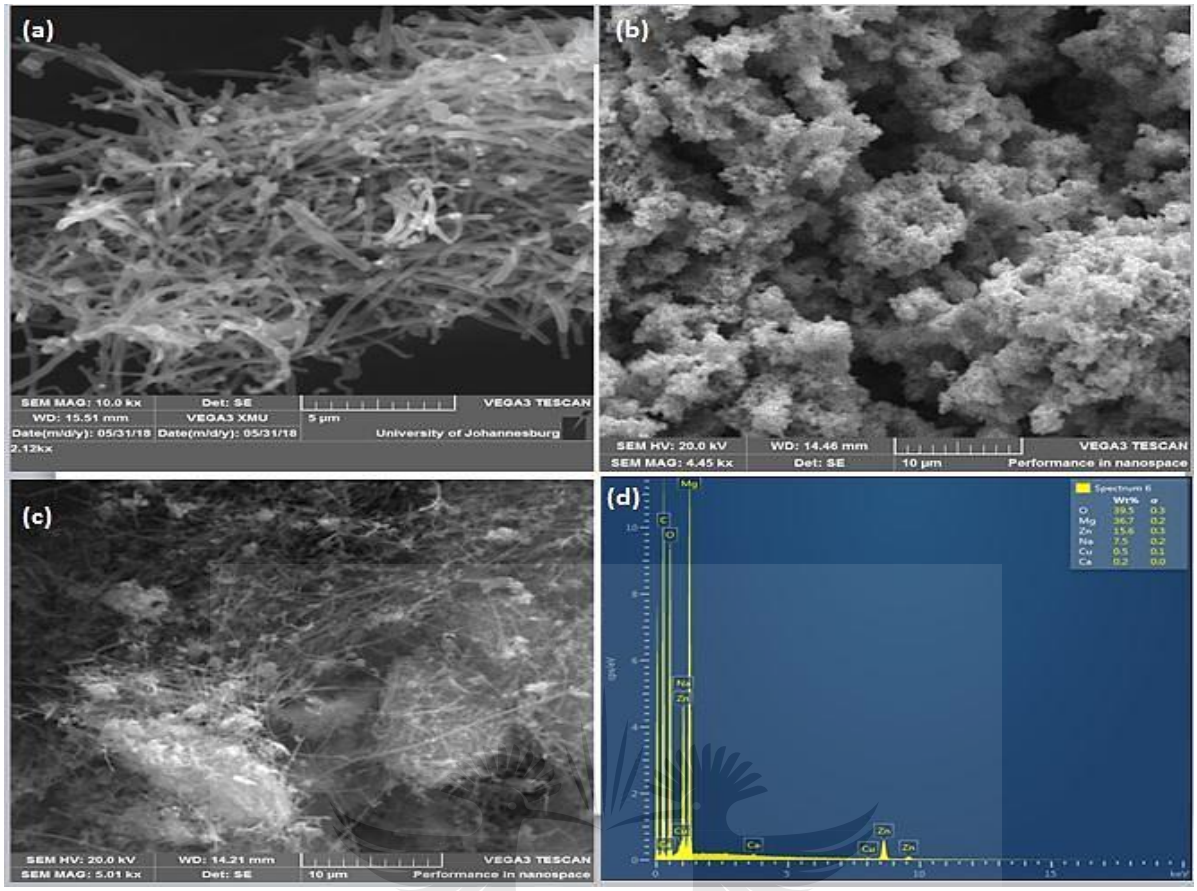

Fig. 4.3: SEM images of (a) CNFs, (b) MgO-ZnO, (c) MgO-ZnO@CNFs and (d) EDX of MgO-ZnO@CNFs nanocomposite

4.3.1.3 Transmission electron microscopy (TEM)

The typical cylindrical and bamboo-shaped carbon nanofibers as illustrated in Fig. 4.4a. The TEM results are consistent with the SEM results and it can be observed that the cylindrical hollow fibers have smooth outer walls that are uniform and made of distinct sandwich of graphite layers. These graphite layers are parallel with respect to the axes of the fibers and display and almost defect free material. The bamboo-shaped fibers on the other hand display some areas of defects and change in diameter in different areas. This is because these types of fibers are composed of multi-walled graphite structures (Kaerkitcha, Chuangchote, & Sagawa, 2016). Fig 4.4b shows how the ZnO-MgO nanoparticles were dispersed onto the CNFs. This figure shows that the ZnO-MgO nanoparticles were significantly larger than the CNFs in diameter. This is also in agreement with BET results that show a significant increase in surface area.

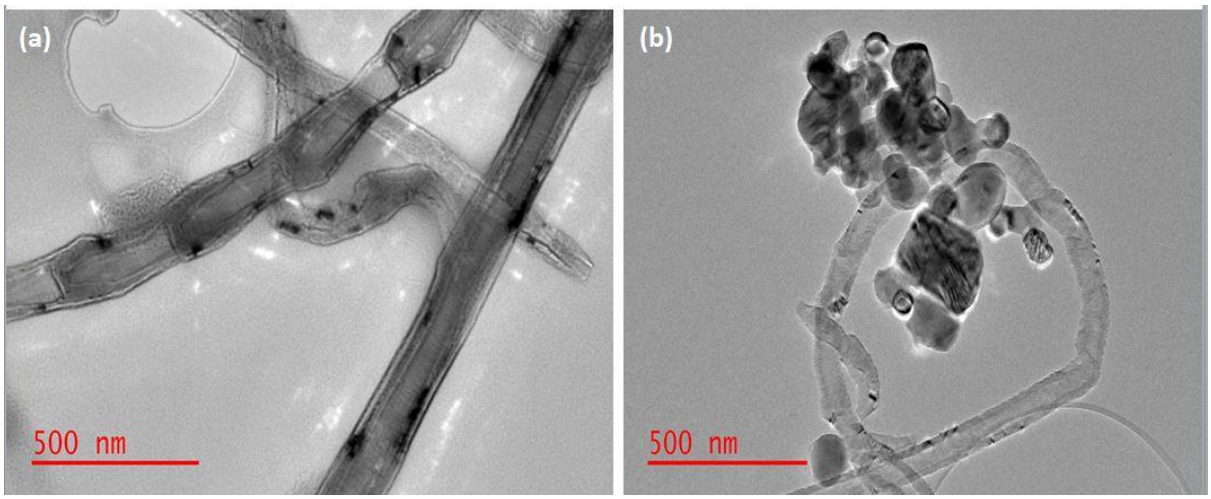

Fig. 4.4: TEM images of (a) CNFs and (b) ZnO-MgO@CNFs taken at 500 nm

4.3.1.3 Brunauer–Emmett–Teller (BET)

The BET surface area of CNFs was found to be 21.1 $m^2\ g^{-1}$ and that of MgO-ZnO@CNFs was found to be 121 $m^2\ g^{-1}$, which means that the incorporation of MgO-ZnO brought more than 500% increase the surface area of CNFs therefore providing more adsorption sites for the analyte. The pore volume of CNFs was found to be 0.12 $cm^3\ g^{-1}$ and that of the MgO-ZnO@CNFs nanocomposite was found to be 0.65 $cm^3\ g^{-1}$ which is also a significant increase that allows more particles of the analyte to be adsorbed on the pores of the composite. The adsorption average pore diameter of the nanocomposite was found to be 21.6 nm.

4.3.1.5 Fourier Transform infrared spectroscopy (FTIR)

Fourier Transform infrared spectroscopy was used to investigate the structural propertied of the nanocomposite. The bands that at 483 and 524 cm^{-1} correspond to the absorption peaks of Zn–O bond and verify the presence of ZnO, the same peaks can be observed on Fig 4.5c. The characteristic peaks observed at 508 and 410 cm^{-1} correspond to the Mg-O bending vibrations present in both Fig. 4.5c and Fig 4.5d. This confirms the presence of MgO in the nanocomposite. The band at 3132, 3426, 3445 and 3428 cm^{-1} arise from the stretching and bending of the O-H group present in the moisture absorbed from the atmosphere. The peaks at 1775 and 1448 cm^{-1} arise from the stretching of C=C and C–C bonds respectively which corresponds to CNFs. These peaks can be observed in Fig 4.5d but with a slight shift to 1636 and 1410 cm^{-1} respectively. This shift towards higher wavenumbers is due to the decrease in mass of CNFs in the nanocomposite.

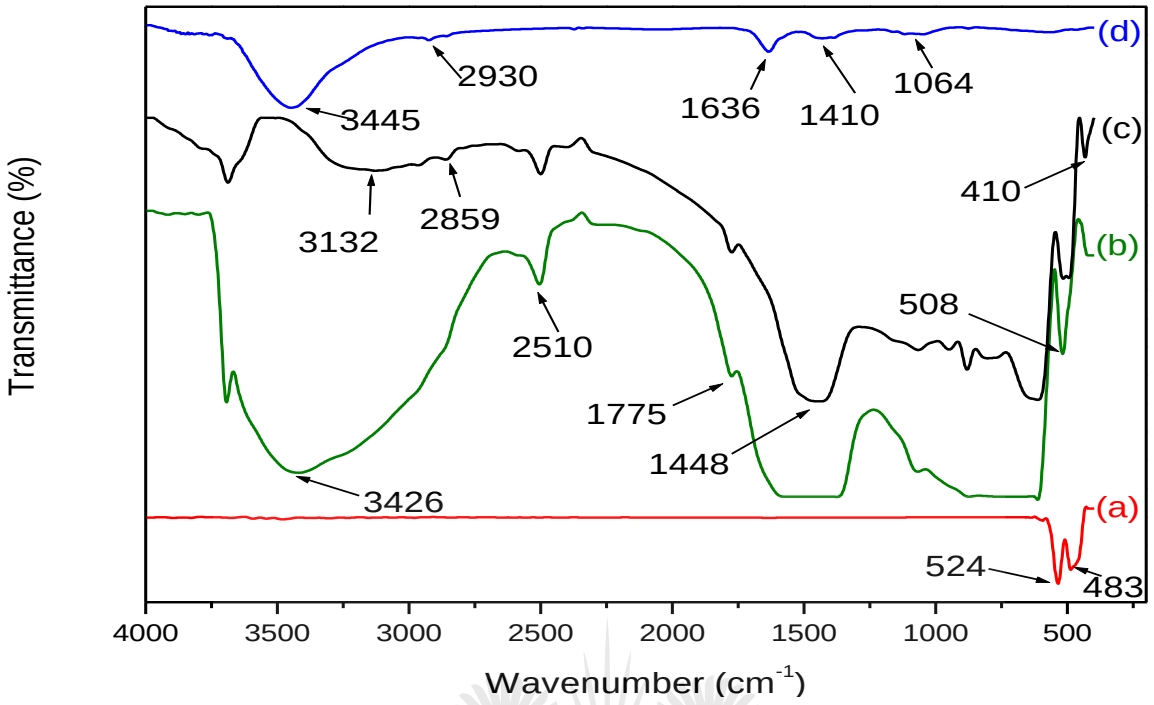

Fig. 4.5: FTIR spectrum of (a) ZnO, (b) MgO, (c) MgO-ZnO@CNFs and (d) CNFs

4.3.2 Optimization of separation and preconcentration method

4.3.2.1 Two level fractional factorial design

In order to achieve optimum conditions, two level fractional factorial design with 12 experiments was conducted to investigate the effect of eluent volume (EV), mass of adsorbent (MA), and pH on the recovery (%R) of CBZ after adsorption as presented in the Table 4.1. The results in Table 4.1 were investigated statistically and the analysis of variance (ANOVA) results were presented in the form Pareto chart (Fig. 4.6). As seen, sample pH, extraction time were significant at 95% confidence level. These findings suggested that mass of adsorbent (MA) and sample pH require further optimisation. Eluent volume was as added as the third parameter. Therefore, central composite design was be used for further optimisation of the three factors.

Table 4.1**:** Optimization parameters and their corresponding values for dispersive solid phase microextraction of CBZ

Experiment No	EV/uL	MA/mg	ET/min	pH	%R
1	500	20	5	4	22.2
2	100	20	5	9	42.5
3	500	60	5	9	48.5
4	1000	60	5	4	57.9
5	500	20	30	9	29.2
6	1000	20	30	4	24.9
7	500	60	30	4	40.5
8	1000	60	30	9	86.2
9 (C)	750	40.	17.5	6.5	95.7
10 (C)	750	40	17.5	6.5	95.4
11 (C)	750	40	17.5	6.5	95.1
12 (C)	750	40	17.5	6.5	95.8

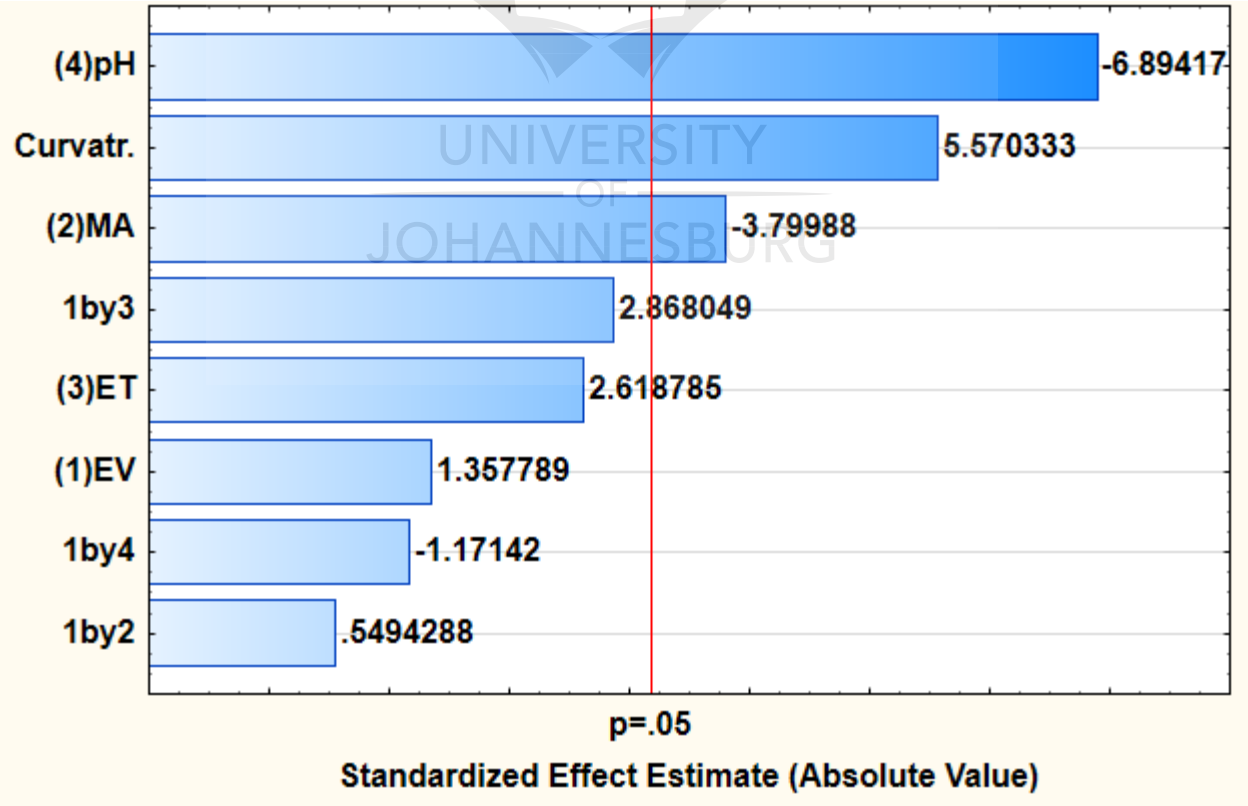

Fig. 4.6: Pareto chart of standardized effects for variables in the preconcentration of CBZ.

4.3.2.2 Response surface methodology (RSM)

Response surface methodology (RSM) provides better knowledge regarding the interactions between individual variables. It also allows the achievement optimal conditions for each investigated variable that will lead to attainment of the maximum analytical response (Asfaram, Ghaedi, Goudarzi, & Soylak, 2015). The three-dimensional response surfaces showing the combined effects of two factors on the analytical response at a constant and fixed level of other variables (central point) are presented in Fig. 4.7. As seen in Fig 4.7(a) and (c), at MA values higher than 20 mg the %R increased and the maximum analytical response was obtained when sample pH was 6-7. Fig. 4.7b shows the desorption of the CBZ analytes from the surface of MgO-ZnO@CNFs nanocomposite with respect to the pH increased with increasing pH and eluent volume. Fig. 4.7a and b indicate that at pH of between 6 and 7, about 100% of CBZ is adsorbed and recovered. The %R decreased as the pH is lower or higher than 6-7. This is due to the protonation and deprotonation of surface functional groups of CBZ as well as the MgO-ZnO@CNFs nanocomposite.

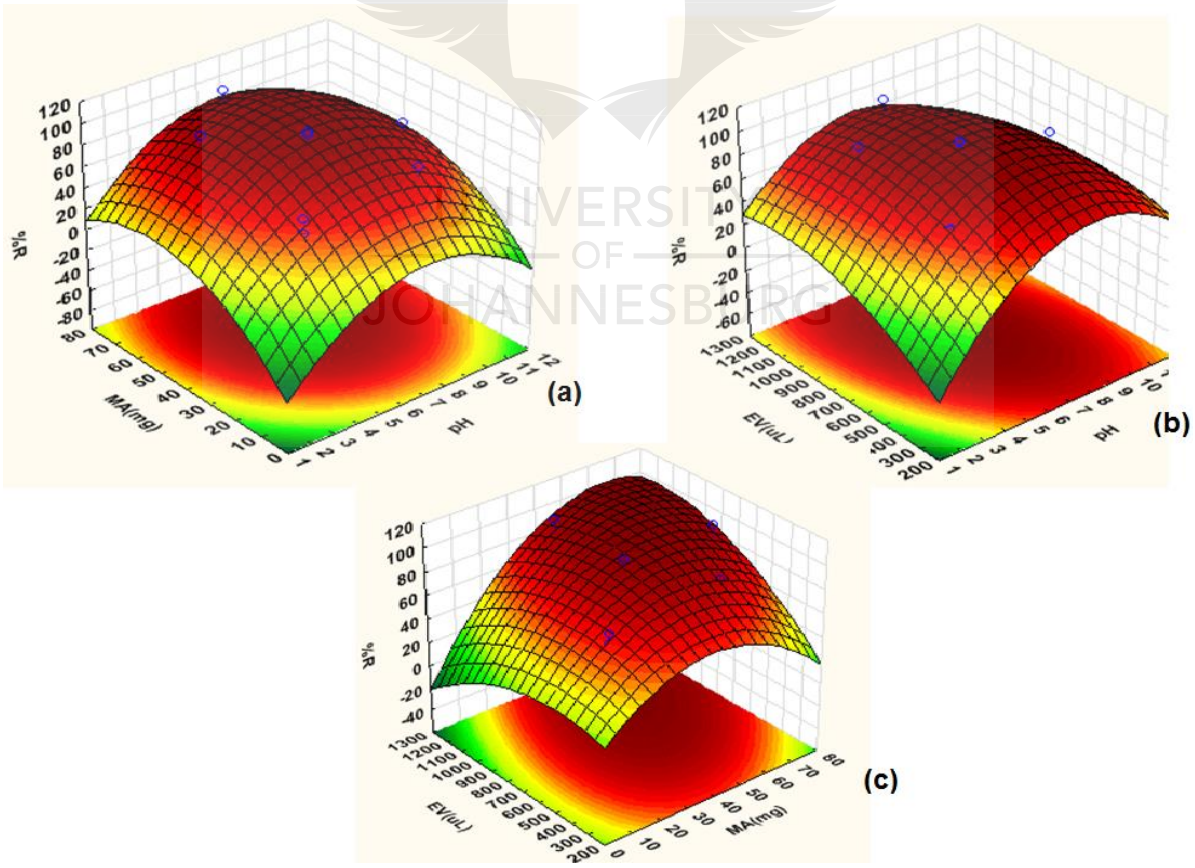

Fig. 4.7: Response surface and contour plots of the combined effects of (a) mass of adsorbent and pH, (b) eluent volume and pH and (c) eluent volume and mass of adsorbent.

The optimisation of the investigated parameters was further investigated using desirability function (Fig. 4.8). The lines observed in Fig. 4.8 demonstrate the ranges of parameters with the exact values of each factor and this information was obtained from the RSM model (Asfaram, Ghaedi, Goudarzi, Soylak, & Langroodi, 2015). The variation of %R with respect to the RSM model variables is described by the bottom lines while the vertical lines demonstrate that sample pH, mass of adsorbent, eluent volume were optimum at 6.5, 74 mg and 1170 µL. respectively. These conditions led to a maximum of 102% with desirability of 1.00. The model was validated experimentally and the experimental value (N = 6) and predicted value of CBZ recovery were 99.7 ± 1.1% and 102%, respectively suggesting the validity and applicability of the RSM model.

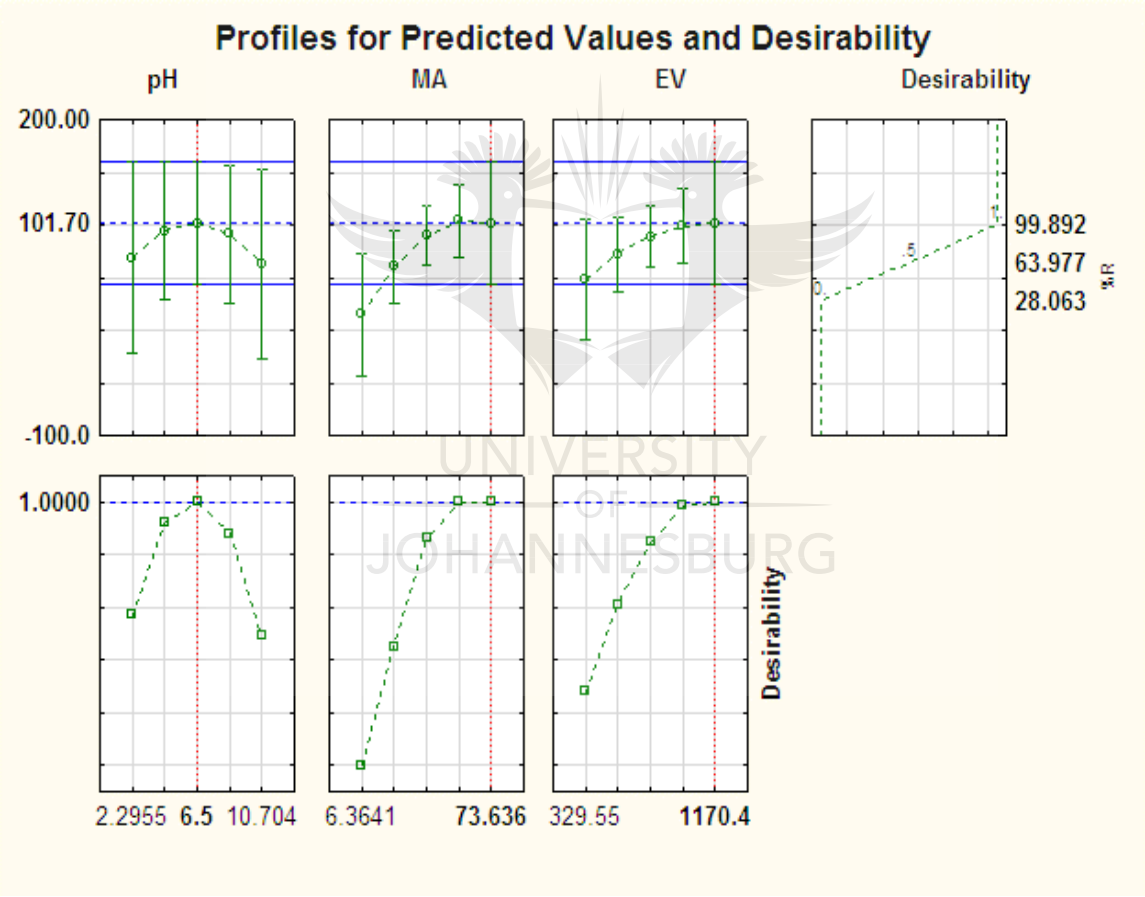

Fig. 4.8: Profiles for experimental and predicated values and desirability function for the recovery of CBZ.

4.3.3 Analytical performance of the UA-DSPE/HPLC-DAD method

Under optimum conditions, the analytical performance of the proposed UA-DSPME/HPC-DAD method for preconcentration and determination of CBZ was evaluated. Several

parameters such as the linear range, precision expressed as relative standard deviation (RSD), limit of detection (LOD), limits of quantification (LOQ) and preconcentration factor were assessed and the results are tabulated in Table 4.2. The LOD and LOQ were calculated as three or ten times standard deviation (n = 10) of lowest concentration of the calibration curve after preconcentration. The UA-DSPME/HPC-DAD method was found to have a linear dynamic range with correlation coefficient of 0.9922. The repeatability was evaluated for ten independent measurements for 100 ng mL^{-1} CBZ concentration performed the same way in one day and the reproducibility was for five working days. As seen, the relative standard deviations (%RSD) were less than 5% and this implied that the method had good precision. Finally the preconcentration factor (PF) was calculated as the ratio of the slopes after and before preconcentration.

Table 4.2: Analytical figures of merit of the applied UA-DSPME/HPLC-DAD method for preconcentration of CBZ.

Analytical figures of merit	**Values**
Regression equation before preconcentration	$y = 0.073x + 6.7261$
Regression equation after preconcentration	$y = 35.735x + 12.972$
Linear range (ng mL^{-1})	0.29-800
Limit of detection (LOD) (ng mL^{-1})	0.08
Limit of quantification (LOQ) (ng mL^{-1})	0.29
Repeatability (RSD, %) (N = 10)	1.4
Repeatability (RSD, %) (N = 10)	4.2
Preconcentration factor (PF)	490

4.3.4 Validation and Application

The accuracy of the UA-DSPME/HPLC-DAD procedure was evaluated using a wastewater samples spiked and unspiked from Daspoort (Pretoria, Gauteng, South Africa) wastewater treatment plant. The water samples were spiked with two different concentrations as shown in Table 4.3. As seen, the recoveries were between 97.8% and 102% with %RSD less than 5 %. These results suggested that CBZ can be quantitatively recovered from the real samples using the developed method. In addition, the results show the ability of UA-DSPME/HPLC-DAD

method to extract, preconcentrate and determine the amount of CBZ in real wastewater samples.

Table 4.3: Recoveries of CBZ from wastewater samples spiked at two levels (100 ng mL^{-1} and 500 ng mL^{-1}) using the with the proposed UA-DMSPME/HPLC-DAD method, n = 3

Sample	**Added (ng mL^{-1})**	**Found (ng mL^{-1})**	**Recovery**
Effluent 1	0	574±8	-
	100	674	99.7
	500	1083±15	102
Influent 1	0	836±8	
	100	934±12	97.8
	500	1430±18	99.1

4.4 CONCLUSION

An efficient ultrasound assisted dispersive solid phase microextraction was developed as sample pretreatment method for extraction and preconcentration of carbamazepine in wastewater samples prior to analysis by HPLC-DAD. The optimum conditions for extraction and preconcentration of CBZ from wastewater samples were obtained using a response surface methodology based on central composite experimental design. The analytical results obtained demonstrated that the MgO-ZnO@CNFs nanocomposite as an adsorbent was suitable for extraction CBZ. Attractive features of the method included low LOD, relatively short analysis, low consumption of sample and adsorbent, reasonably high preconcentration factor, wide linear dynamic range, and excellent recoveries with high accuracy and precision.

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CHAPTER 5:
GENERAL CONCLUSIONS, RECOMMENDATIONS AND FUTURE PERSPECTIVE

6.1 GENERAL CONCLUSION

Pharmaceuticals and hormones play an important role in wellbeing of human and animals, growth and reproductive functioning. However, their increasing production and use has resulting to environmental pollution. The source of these compounds include municipal and industrial wastewater effluent, agricultural and terrestrial runoff, accidental release and excrements of living organisms (humans and animals). Even though emerging contaminants are present at trace to ultra-trace levels, their adverse effects on environmental and human health. Therefore, unceasing efforts have been dedicated develop simple, rapid, and efficient analytical methods for the determination of emerging contaminants in environmental matrices.

In this work two nanometer sized metal oxides coated carbon nanofibers composites (Fe_3O_4-Al_2O_3@CNFs and MgO-ZnO@CNFs) were synthesised and characterised by FTIR, XRD, SEM/EDS, TEM and BET. Ultrasound assisted dispersive magnetic solid phase microextraction (UA-DMSPME) based on Fe_3O_4-Al_2O_3@CNFs as the adsorbent material was developed and used for preconcentration of E2. Ultrasound assisted dispersive solid phase microextraction (UA-DSMPE) utilizing MgO-Zno@CNFs adsorbent was applied for the preconcentration of CBZ from environmental samples prior to HPLC determination. Several operational parameters affecting the preconcentration procedures were first investigated using fractional factorial design approach as a screening then further optimisation was conducted using response surface methodology based on Box-Behnken and central composite design. The developed methods, that is, UA-DMSPME/HPLC-DAD and UA-DSPME/ HPLC-DAD were successfuly applied in the analysis of E2 and CBZ in wastewater samples. The both displayed high extraction efficiency, low detection limits, wide linear ranges, reasonably high preconcentration factors and excellent recoveries with high accuracy and precision. Other attractive features of the methods included relatively short analysis, low consumption of sample and adsorbent.

6.2 RECOMMENDATIONS AND FUTURE PERSPECTIVE

The nanocomposites prepared and applied in this study proved to be suitable alternative adsorbents that can be used in modern flow-based SPE techniques. In addition, the materials displayed a potential for the sample handling and pretreatment of complex samples such as wastewater. Therefore, further work will be directed towards improvement of the experiments as well as the preparation of other carbon nanomaterials and modifying their surface properties with environmentally friendly nanometal oxides. The materials will be then used in the development of novel extraction approaches for analysis of emerging pollutants in the environmental matrices.

APPENDIX

Table 3A-1. Materials with their corresponding angles and phases.

Material	Angle	Phase
CNFs	26.38º	(002)
Fe_3O_4	30.36º	(220)
	35.77º	(311)
	43.47º	(400)
	57.51º	(511)
	63.17º	(440)
Al_2O_3	25.28º	(012)
	34.72º	(104)
	37.34º	(110)
	42.83º	(113)
	45.63º	(202)
	56.77 º	(116)
	59.90º	(024)
	67.34º	(300)
	73.29º	(104)
	76.16º	(119)
	83.19º	(223)

Table 4A-1. Materials with their corresponding angles and phases.

Material	**Angle**	**Phase**
CNFs	25.94º	(002)
MgO	37.15º	(111)
	43.13º	(200)
	62.51º	(220)
	74.86º	(311)
	78.80º	(222)
ZnO	31.82º	(100)
	34.48º	(002)
	36.30º	(101)
	47.59º	(102)
	56.63º	(110)
	62.90º	(103)
	66.41º	(200)
	67.99º	(112)
	69.12º	(201)
	72.61º	(004)
	76.98º	(202)
	81.42º	(104)
	89.65º	(203)